each brain sample. Four intact male Swiss mice, which were offspring of mice obtained from Charles River Breeding Laboratories, were used for each data point in the first experiment and 2 mice per data point in the second experiment.

BEHAVIORAL EXPERIMENTS AND RESULTS

EFFECTS OF ACTH 4-10-L-Phe-7 ON RETENTION EXPERIMENTS 1–3

Experiment 1: Dose Response

The purpose of the experiment was to determine the dose range that might facilitate retention. ACTH 4-10-L-Phe-7 (ACTH-L) was administered 30 min after passive avoidance training. A low shock level of 0.30 mA was chosen so that controls would show poor performance and thus improvement of retention could be measured. As a further means of setting the proper level of training, we retained only those mice with latencies-to-enter by latencies-to-escape of 2 sec x 1 sec, 2 x 2, 2 x 3, 3 x 1, or 3 x 2 (values rounded to the nearest second). These latency values are very important since they determine the degree of learning [11]. ACTH-L was administered at 0, 0.1, 0.3, 1.0 and 3.0 mg/kg. The 0.1 and 0.3 mg/kg doses are values similar to those used by de Wied and his associates.

Results

The results summarized in Fig. 1 show that for this passive avoidance task, the 0.1, 0.3 and 1.0 mg/kg doses of ACTH-L facilitated retention significantly and equally, that is, these groups had all showed about 80% retention, whereas the group receiving no ACTH-L showed only 27% (Fig. 1). There is some indication that too much of the peptide produces less than optimal facilitation of memory formation since the 3.0 mg/kg showed 50% retention.

Experiment 2: Interaction of Dose and Time of Administration

The purpose of this experiment was to determine if (a) ACTH-L could produce a better dose response curve with a decrease in training strength from that used in Experiment 1, and (b) if the effect of ACTH-L was dependent on the time of drug administration after training. The mice were trained (shock intensity 0.30 mA) and injected in the same manner as in Experiment 1 except that the latency-to-enter had to be 2 sec and the latencies-to-escape 1 or 2 sec. This shifted the distribution of training values toward those producing somewhat poorer retention; that is the controls were amnesic. Thus the test should be more sensitive to the effects of ACTH-L. The N per group was 20.

Results

The dose response portion of the ACTH-L curve over which retention improved was between 0.03 and 0.1 mg/kg. The dose of 0.3 mg/kg seemed to produce the optimal level of improvement in retention (Table 1). However, the effective dose range was still 0.1 to 1.0 mg/kg. The effect of ACTH-L on retention was dependent on the time of its administration. Administration of the peptide at 30 or 60 min after training had similar effects on retention. But ACTH-L was less effective in improving retention when administered 90 min after training and an additional loss of effectiveness was observed when administration occurred

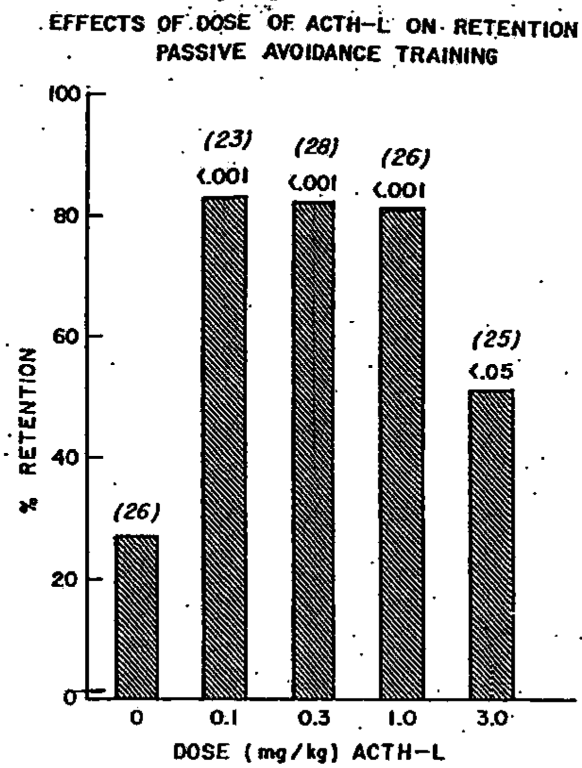

FIG. 1. The effects of dose of ACTH-L on retention for passive avoidance training. The ACTH was administered 30 min after training. Retention was tested one week later. N/group was between 23 and 28.

240 min after training (Table 1). The decrease in effectiveness of the higher ACTH-L doses was found again. In addition, this modified training procedure was an improvement over the procedure previously used since it made it possible to demonstrate that a dose of 0.3 mg/kg was optimal.

Experiment 3: ACTH-L on Retention using T-Maze Active Avoidance

The purpose of this experiment was to test if ACTH-L would have the same effect on memory formation for an active avoidance task as it did in the passive avoidance task. The mice were given 4 training trials on the T-maze using a muffled door bell buzzer as CS. ACTH-L was administered within 1 min after training at doses of 0.3 or 3.0 mg/kg. A saline-injected group served as control. The N per group was 10.

Results

Administration of ACTH-L immediately after active avoidance training improved retention scores both as measured by the percentage of mice requiring more than 3 trials to make the first avoidance response i.e., amnesic mice (Fig. 2B), and by the number of trials for the first avoidance (Fig. 2A). Again as in the passive avoidance task, the higher dose was less effective than the lower dose of ACTH-L.

TABLE 1

AMNESIA AS A FUNCTION OF TIME AND DOSE OF ACTH-L PASSIVE AVOIDANCE TASK

Time of Injection after training (min)	Dosage in mg/kg 0.0	0.03	0.1	0.3	1.0	3.0
	% Amnesic Mice*					
30	80	60	30	15	35	55
60	80	60	25	20	50	60
90	85	75	50	40	50	75
240	80	80	75	65	75	75

*N = 20/group.

EFFECTS OF ACTH 4-10-D-Phe-7 ON RETENTION
EXPERIMENTS 4–5

Experiment 4: Interaction of Dose and Time of Administration of ACTH-D

The purpose of the experiment was twofold: (1) to determine what doses of ACTH 4-10-D-Phe-7 (ACTH-D) would affect retention test scores and (2) to determine the influence of time of administration on the effect. The mice were trained on one-trial passive avoidance. In Experiment 2 it was necessary that the control animals would be only minimally trained so that facilitation of memory could be demonstrated. In this experiment control animals need to be well trained so that impairment of memory formation by ACTH-D could be shown. One way of increasing training strength is to raise the level of shock intensity. For this reason shock intensity was 0.33 mA rather than 0.30 mA as in Experiment 2. ACTH-D was administered 30, 60, 90, or 240 min after training at doses of 0.3, 1.0 or 3.0 mg/kg. Saline was administered as a control for the stress of the injection. We retained only animals with latencies-to-enter of 2 sec and latencies-to-escape of 2 sec (when rounded off to the nearest second). The N per group was 20.

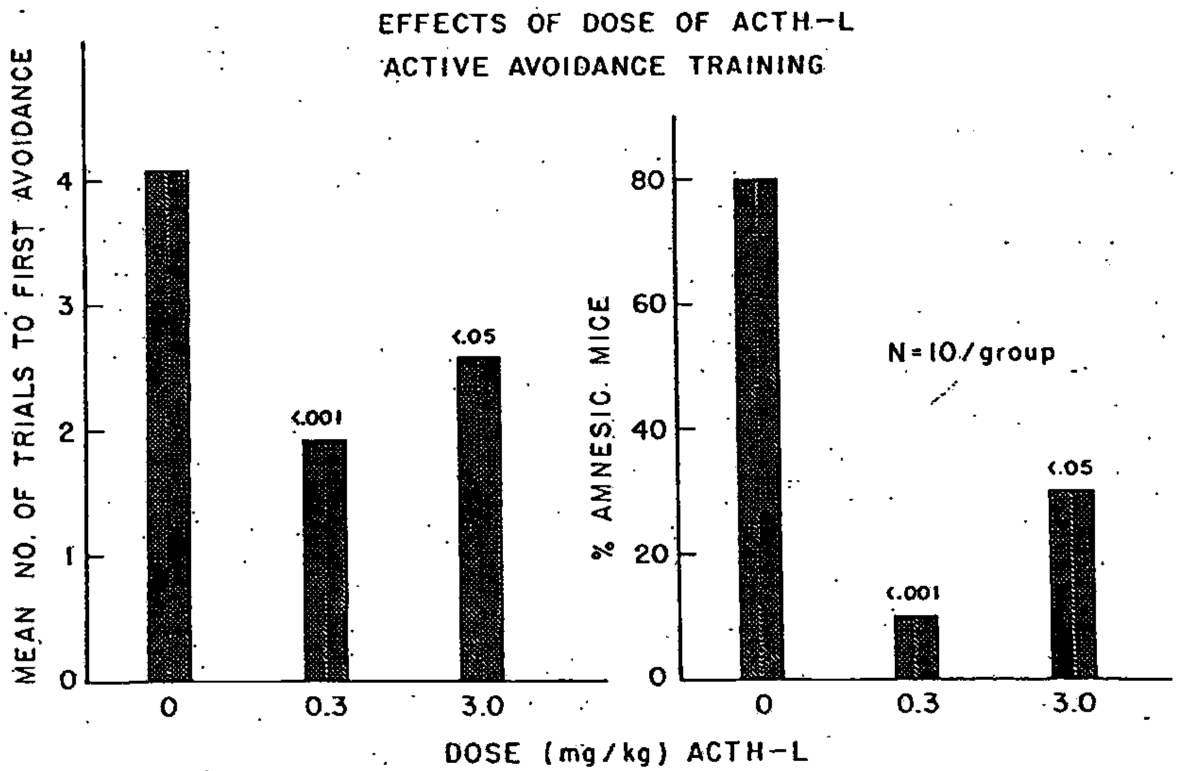

FIG. 2. Effect of dose of ACTH-L on active avoidance training. ACTH-L was administered immediately after training. A, (left) Mean number of trials to first avoidance; B, (right) Percentage of mice requiring more than 3 trials to make first active avoidance response.

Results

All three doses of ACTH-D were effective at reducing retention scores and thus increasing amnesia when administered 30 or 60 min after training. There is a trend under these conditions of training for the high dose of ACTH-D to be more effective than the low dose (Table 2). As was found with ACTH-L in Experiment 2, ACTH-D exerted a greater effect when injected 30 or 60 min than when injected 90 or 240 min after training.

TABLE 2

AMNESIA AS A FUNCTION OF TIME AND DOSE OF ACTH-D PASSIVE AVOIDANCE TASK

Time of Injection after training (min)	Dosage in mg/kg 0.0	0.3	1.0	3.0
	% Amnesic Mice*			
30	20	55	65	75
60	15	55	70	70
90	15	30	35	40
240	20	10	30	45

*N = 20/group.

Experiment 5: ACTH-D Effect on T-Maze Active Avoidance

The purpose of the experiment was to determine if ACTH-D would have the same effect on retention for an active avoidance task as it did in a passive avoidance task. In Experiment 3 we examined the facilitating effect of ACTH-L on an active avoidance task, therefore it was necessary for controls to be minimally trained so only 4 training trials were given. In this experiment we are looking for impairment of memory, therefore well-trained controls were needed. For this reason the mice were given 5 training trials with a loud door bell buzzer as CS. In order to control the degree of learning a training performance criterion was imposed. Animals were not included if they made an avoidance response during original training or if they failed to make one correct escape response. ACTH-D was administered within 1 min after training at doses of 0.0, 0.3, 1.0 or 3.0 mg/kg. The retention test, as in all experiments of this series, was one week after training. The N was 20 per group.

Results

ACTH-D at 1.0 or 3.0 mg/kg significantly impaired retention; that is the percentage of amnesic mice increased (Table 3). As with Experiment 4, a significant difference was not found between 1.0 and 3.0 mg/kg doses although there is indication of greater effect with the larger dose (compare Tables 2 and 3).

The mice which made avoidance responses during the original training were analyzed separately to see if ACTH-D would still have an effect (Table 4). Interestingly, this seems to have brought out the differences in dose response. ACTH-D at 3.0 mg/kg caused more amnesia. Owing to the small N's no differences were significant but the group given 3.0 mg/kg differed from the control group at approximately $p<0.06$.

TABLE 3

AMNESIA AS A FUNCTION OF DOSE OF ACTH-D ACTIVE AVOIDANCE TASK

Dose in mg/kg	0.0	0.3	1.0	3.0
% amnesic mice*	20%	40%	60%	65%
p value from 0.0		NS	0.05	0.02

*N = 20/group. Mice not making the first avoidance response in 3 trials or less were scored as amnesic.

TABLE 4

AMNESIA AS A FUNCTION OF DOSE OF ACTH-D SELECTED ANIMALS*—ACTIVE AVOIDANCE TASK

Dose in mg/kg	0.0	0.3	1.0	3.0
N	10	8	7	8
%Amnesia	10%	12%	14%	50%

*Only those animals which made an avoidance during the original training were used. Amnesic mice defined as in Table 3.

EFFECTS OF ACTH ON AMNESIA PRODUCED BY ANISOMYCIN: EXPERIMENTS 6–9

Experiment 6: Effects of ACTH-L

Protein synthesis inhibition by anisomycin (Ani) was used to cause amnesia in this experiment. In some of the mice ACTH-L was administered shortly after training (30 min) to determine if the ACTH-L would block the amnesia. Other groups received more injections of Ani to test if longer durations of inhibition of protein synthesis would block the anti-amnesic effect of ACTH-L.

Mice were trained on passive avoidance at a footshock of 0.36 mA. This is a relatively high footshock and produces strong training. Ani was administered 15 min prior to training and again at 1-3/4 hr after training. Some groups received a third injection of Ani 3-3/4 hr after training. Saline-injected mice served as controls. Saline, 0.3 or 3.0 mg/kg of ACTH-L was administered 30 min after training. N per group was 20.

Results

As expected, two or three successive injections of Ani caused significantly greater amnesia than was found in the saline control (80 and 85% vs 15%). An injection of ACTH-L at the dose of 3.0 mg/kg significantly reduced this amnesia in mice given 2 successive injections of Ani but not in those given 3 successive injections of Ani (Fig. 3). The effect of 0.3 mg/kg ACTH-L, while significant, did not cause such a large drop in the percent amnesia. ACTH-L did block the amnestic effect of two successuve injections of Ani but increasing the number of injections (and thus the duration of inhibition) blocked the anti-amnestic effects of the ACTH-L.

The high dose of ACTH was more effective at reducing the level of amnesia than the lower dose. However, an additional injection of Ani which prolongs inhibition of protein synthesis by 2 hr at 80% or more blocked the anti-amnestic effect of the ACTH-L.

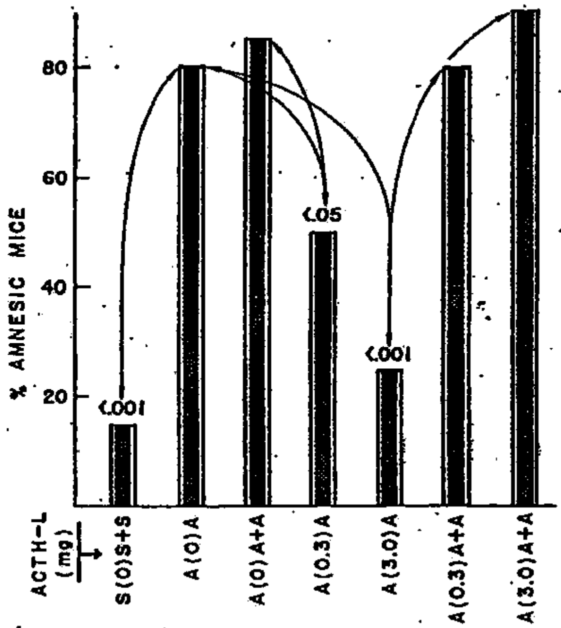

FIG. 3. Effects of Anisomysin (A) and Dose of ACTH-L on retention for a passive avoidance task. A was administered 15 min prior to training and subsequently at 2 hr intervals as indicated. ACTH or saline (S) was administered 30 min after training. ACTH-L overcame the amnestic effects of 2 doses of A, but not of 3 doses. The 3.0 mg/kg dose of ACTH was more effective than 0.3 mg/kg.

Experiment 7: Effects of Number of Injections of ACTH-L

The purpose of this experiment was to determine if additional injections of ACTH-L would block the effect of additional injections of Ani as observed in Experiment 6. The conditions of training were the same as in Experiment 6. The injection groups are given in Fig. 4. Ani or saline were administered 15 min prior to training and 1-3/4 hr after training. Some groups received a 3rd injection 3-3/4 hr after training. ACTH-L or its control injection (saline) was administered 1/2 hr or 1/2 h and 2-1/2 hr after training, ACTH-L was administered at a dose of 3.0 mg/kg. N per group was 10.

As in experiment 6, two injections of Ani caused significantly more amnesia than did injections of saline. ACTH-L blocked the amnesia in groups receiving only two successive injections of Ani. Neither a single or double injection of ACTH-L blocked the amnesia induced by three successive injections of Ani (Fig. 4). These data, like the time dependent results of Experiment 2, suggest that ACTH-L alters memory formation only when administered within the first hour after training.

Experiment 8: Effects of ACTH-D

Under appropriately high conditions of training the amnestic effect of high levels of protein synthesis inhibition by anisomycin can be reduced so that one can test if another substance will increase amnesia.

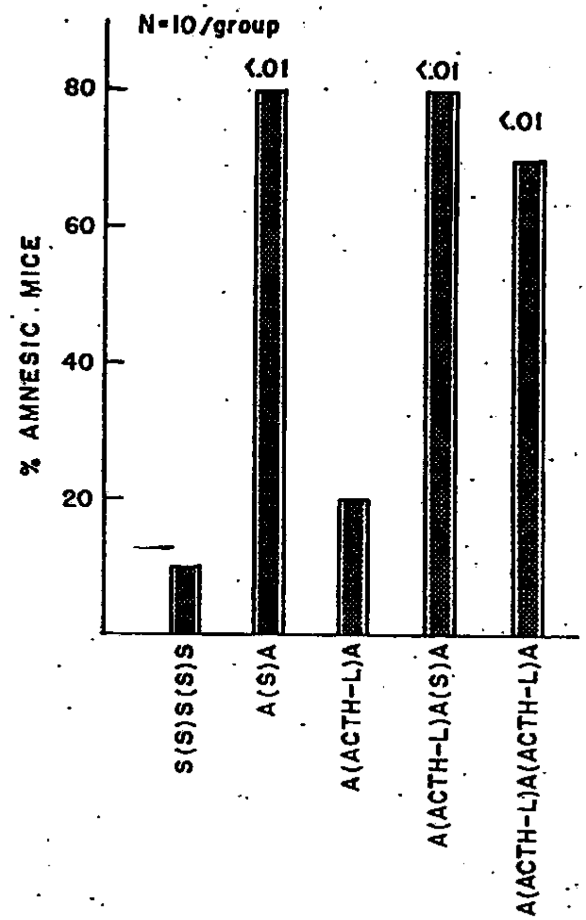

FIG. 4. Effect of number of injections of ACTH-L on amnesia produced by anisomycin: Anisomycin (A) was administered 15 min prior to training on a passive avoidance task and subsequently at 2 hr intervals. ACTH-L or saline (S) was administered 30 min after training and subsequently at 2 hr intervals as shown. ACTH-L did not overcome the amnesia produced by 6 hr of high inhibition of protein synthesis from 3 injections of anisomycin.

Animals were trained on the passive avoidance task. They received two injections of either Ani or saline 15 min prior to training and 1-3/4 hr after training. ACTH-D was administered 30 min after training at 0.0, 0.3, 0.1 or 3.0 mg/kg. The latency-to-enter and escape was 2 sec by 2 sec. The footshock intensity was 0.34 mA. The N per group was 20.

Results

Two successive injections of Ani alone did not cause

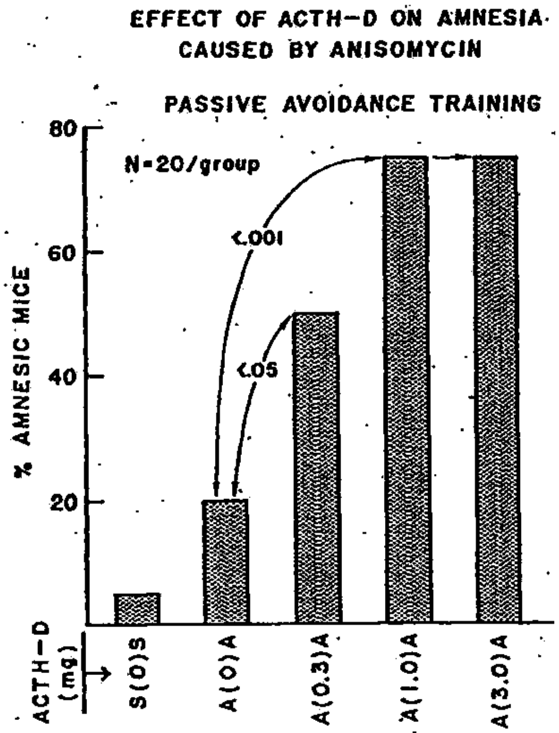

FIG. 5. Effect of ACTH-D and anisomycin on amnesia. With strong training, for a passive avoidance task, anisomycin administered 15 min prior and 1-¾ hr after training caused only a low percentage of amnesic mice. ACTH-D increased the amnesia; high doses were more effective than low doses.

significant amnesia. The groups receiving ACTH-D all differ significantly from the group receiving only [A(ACTH-D 0)A] (Fig. 5). The 3.0 and 1.0 mg/kg doses differ at $p<0.001$. The groups receiving ACTH-D did not differ significantly from each other but a strong indication of the weaker effect of the low ACTH-D dose versus the high is present. The doses 1.0 and 3.0 mg/kg in combination with Ani induced the greatest retention deficit or amnesia.

OPPOSING EFFECTS OF ACTH 4-10-L- AND-D-Phe-7 ON RETENTION

Experiment 9A

The results of Experiments 1 through 8 indicated that ACTH-L facilitated memory formation while ACTH-D impaired memory formation. As a further test of the opposing effects of these peptide fragments, we administered ACTH-L at its optimal dose (0.3 mg/kg) and combined this with various doses of ACTH-D (0.3 to 6.0 mg/kg) to determine how much ACTH-D is required to block the facilitating effects of ACTH-L.

The mice were trained on passive avoidance at a footshock intensity of 0.30 mA. Animals had to have latencies-to-enter of 2 sec and latencies-to-escape of 2 sec to be retained. The ACTH-L, ACTH-L + ACTH-D (combined in one injection), or saline were administered 30 min after training. The doses of the peptides and the N's per group are given in Fig. 6A.

Results

ACTH-L given alone significantly enhanced retention when compared with the saline controls (Fig. 6A). A dose of ACTH-D at 3.0 or 6.0 mg/kg was sufficient to block the effect of ACTH-L. It appeared that 6.0 mg/kg of ACTH-D completely blocked the effect of ACTH-L (0.3 mg/kg).

Experiment 9B

As a further check on whether such a high dose of ACTH-D was required to block the ACTH-L effect on retention, Experiment 9A was repeated except that the footshock was reduced slightly to 0.28 mA. This was done to increase the percentage of amnesic mice in the control group. The groups used are shown in Fig. 6B. Other conditions were as in experiment 9A.

Results

ACTH-L again enhanced retention. In fact the percent difference was similar under the two training conditions (a difference of 45% for Experiment 9A, 58% for Experiment 9B; ACTH-L versus Saline). Under less vigorous training only the 6.0 mg/kg dose of ACTH-D completely blocked the effect of ACTH-L (Fig. 6B).

Experiment 9C

Experiments 9A and 9B showed that large doses of ACTH-D blocked the retention-enhancing effect of the ACTH-L. The results were interpreted to indicate that ACTH-L and ACTH-D have the same site of action for effecting memory formation but have opposing action. An alternate suggestion made at this Conference was that the apparent opposing effects of ACTH-D and ACTH-L were due to the increasing total dose of ACTH. It may be

TABLE 5

EFFECT OF ACTH-L, ACTH-D, AND ANISOMYCIN ON [^{14}C]-VALINE INCORPORATION IN WHOLE BRAIN
% INHIBITION OF VALINE INCORPORATION

Sal or Ani time (min)	75		100		135	
Total ACTH (min)	30		55		90	
Valine Incorporation (min)	15		15		15	
	Sal	Ani	Sal	Ani	Sal	Ani
ACTH none	0	90	0	85	0	73
ACTH-L 2.0 mg/kg	5	90	13	85	10	76
ACTH-D 0.5 mg/kg	7	90	6	88	−3	69

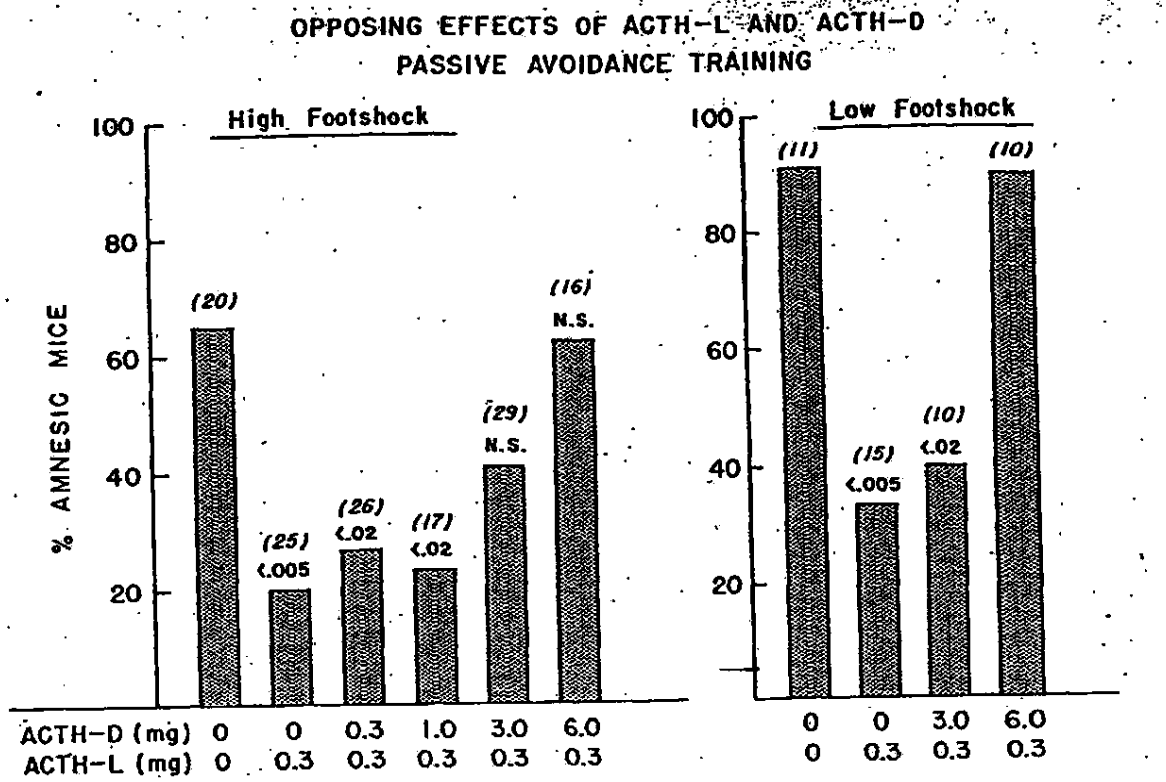

FIG. 6. Interaction of ACTH-D and ACTH-L on retention for a passive avoidance task. Under either condition of strong training (high footshock) (left) or weak training (right), ACTH-D blocked the enhanced retention produced by ACTH-L. Drugs were administered 18 sec after training. N's/group and significance of difference from the control groups are shown above the data bars.

recalled that in Experiment 1, higher dosages of ACTH-L were less effective than lower doses. The purpose of this experiment was to determine if increasing the total amount of ACTH necessarily resulted in increased or sustained high levels of amnesia. The mice were well-trained on passive avoidance using 0.33 mA as in Experiment 4. The experimental groups of mice are given in Table 7. The ACTH or saline was administered 30 min after passive avoidance training. Testing was one week after training. The N per group was 10.

Results

The results show that simply increasing the total amount of ACTH did not result in amnesia. A high percentage of amnesia resulted only when ACTH-D was administered at

TABLE 6

EFFECT OF ACTH-L, ACTH-D AND ANISOMYCIN ON [^{14}C]-VALINE INCORPORATION
% INHIBITION OF VALINE INCORPORATION

	Rest of Brain				Brain Stem			
Sal or Ani time (min)	135		180		135		180	
Total ACTH time (min)	90		135		90		135	
Valine Incorporation (min)	15		15		15		15	
	Sal	Ani	Sal	Ani	Sal	Ani	Sal	Ani
ACTH added								
none	0	68	0	53	0	53	0	38
ACTH-L 0.5 mg/kg	13	71	−13	61	2	64	−1	43
2.0 mg/kg	−1	68			−10	58		
ACTH-D 0.5 mg/kg	−11	76	4	63	−20	61	−8	44
Mannitol 333.3 mg/kg	−11		−12	63	5			49

(−) denotes stimulation.

TABLE 7

AMNESIA AS A FUNCTION OF DOSE OF ACTH-D AND ACTH-L PASSIVE AVOIDANCE TASK

ACTH-D (mg/kg)	ACTH-L (mg/kg)	%Amnesic Mice*
9.0	0	80%
7.0	0	90%
6.0	0	80%
6.0	1.0	50%
6.0	3.0	30%
0	0	30%

*N = 10/group.

dosages of 6.0 to 9.0 mg/kg and in the absence of ACTH-L. When ACTH-L was added to the ACTH-D, amnesia was reduced or blocked (Table 7). For example, those mice receiving ACTH-D, 6.0 mg/kg plus ACTH-L, 3.0 mg/kg received as much total peptide as mice administered ACTH-D, 9.0 mg/kg. Combining the peptides resulted in only 30% amnesia, while 9.0 mg/kg ACTH-D alone yielded 80% amnesia. When compared to the group given 7.0 mg/kg of ACTH-D, the group given 6.0 mg/kg of ACTH-D plus 1 mg/kg of ACTH-L also showed decreased amnesia (90% vs 50%). The results show that with respect to memory formation, the action of ACTH-L and ACTH-D are opposing rather than additive. In addition, it is likely that they have the same site of action.

BIOCHEMICAL RESULTS

The results of Biochemical Experiments 1 and 2 indicate that ACTH-L or ACTH-D, when administered subcutaneously to intact mice, did not alter cerebral protein synthesis by more than 10% within 2-1/4 hr (Tables 5 and 6). Furthermore, these peptides did not significantly modify the inhibition caused by anisomycin. Mannitol, which had been used to solubilize the ACTH peptides for the biochemical experiments, by itself caused a slight increase of valine incorporation. It should be emphasized that mannitol was not used in the behavioral experiments since it introduced side effects there also.

DISCUSSION

ACTH Effects on Memory Formation

The finding of primary importance in these experiments was that ACTH-L, a peptide fragment of ACTH, administered shortly after training can influence subsequent retention test performance and thus aid memory formation (Experiments 1–3). ACTH-D, containing the unnatural isomer of phenylalanine, impairs memory formation (Experiments 4–5). These effects do not appear to be proactive since it was found that the effect of the ACTH peptides was time dependent. Such peptides can have an effect on retention test performance when administered shortly prior to testing [2, 5, 6, 19, 29] and for the most part these effects are consistent with those found in our studies.

The results confirm previous reports that ACTH 4-10-L-Phe-7 and ACTH 4-10-D-Phe-7 have opposite effects on retention for an active avoidance task [1, 19, 29] and for an appetitive task [15]. Gray and Garrud [17] have reported that ACTH-L and ACTH-D given during acquisition had opposite effects during the non-rewarded extention test; the L-peptide blocked the effect of the non-reward, the D-peptide accelerated the extinction. ACTH-L consistently enhanced retention in active and passive avoidance tasks (Experiments 1, 2, 3). ACTH-D impaired retention in active and passive avoidance (Experiments 4, 5). However, Greven and de Wied [19] reported that ACTH 1-10-L-Phe-7 did not show the anticipated reversal of the effects of ACTH 1-10-L-Phe-7 for a passive avoidance task. Consistent with the opposing actions of the two peptides was the finding that ACTH-L acted as an anti-amnestic agent (Experiment 6) and ACTH-D potentiated amnesia caused by brain protein synthesis inhibition (Experiment 8).

Effective Dose Range of the ACTH Peptides

The effective dose range of the peptides was similar to that reported for extinction test studies [19, 24, 29]. ACTH-L was effective over a dose range of 0.1 to 1.0 mg/kg or 3 to 30 μg per 35 gm mouse. The optimal dose was 0.3 mg/kg or 10 μg per mouse. The ACTH-D effective dose range was somewhat higher than reported for extinction studies. Doses from 0.3 to 3.0 mg/kg were effective and 1.0 mg/kg or 30 μg/mouse seemed to be the most effective dose. Extinction studies have shown that the effective dose range of the L- and D-ACTH peptide fragments is about the same. However, when administered different ratios of the peptides after training, ACTH-D seemed to have significantly less affinity for the receptor site than the L form, since it took a ratio of ACTH-D to ACTH-L of at least 10 to 1 to block ACTH-L facilitation (Experiment 9A and 9B). It is also possible different receptors are involved with the ACTH-D receptor being less active. A third possibility would be differences in effective concentration of the two drugs at the active sites. De Wied has presented evidence that different receptor sites exist for the two forms of the peptide [6].

Anti-amnestic Studies

We have shown that anisomysin administered at 2 hr intervals will maintain a high level of inhibition of protein synthesis [12]. There is an interaction between the strength of training as determined by several factors including the shock intensity, duration of shock, etc., and the duration of inhibition required to produce amnesia [11–13]. We have recently shown that a number of stimulants and depressants, which act by a variety of mechanisms, can modify the amnesia produced by anisomysin (Flood *et al.*, submitted to *Behavioral Biology*). In the present series of experiments, it was found that ACTH-L and ACTH-D can also modify anisomycin-induced amnesia. ACTH 4-10-L-Phe-7 was found to block anisomycin-induced amnesia when the peptide was injected 30 min after training. However, extending the duration of inhibition by an additional 2 hr by a 3rd injection of Ani re-established the amnesia (Experiment 6). An additional injection of the peptide was not able to block amnesia when three successive anisomycin injections were used (Experiment 7).

ACTH-D increased a partial amnesia caused by anisomycin after strong training. We would like to caution against an interpretation that the anti-amnestic effect of ACTH-L suggests that anisomycin causes amnesia by interfering with ACTH function or vice versa. In this series of experiments, we were unable to show that ACTH-L or

ACTH-D had more than a slight generalized effect on protein synthesis as measured in large brain areas. In addition, neither ACTH-L nor ACTH-D appeared to change significantly the overall inhibition of protein synthesis produced by anisomycin. In interpreting the effects of ACTH peptides on memory other results that we have obtained should also be considered: (a) ACTH-L improves retention in poorly trained mice; (b) stimulants including d-amphetamine, strychnine, picrotoxin, caffeine, nicotine, and bicuculine all blocked amnesia induced by anisomycin and the amnesia could be regained by extending the duration of inhibition of protein synthesis (Flood *et al.*, ibid, and unpublished observations); (c) dexamethasone and hydrocortisone could block amnesia induced by inhibition of protein synthesis; (d) depressants, including sodium phenobarbital, chloral hydrate, chloropromazine, meprobamate, potentiated anisomycin-induced amnesia (Flood *et al.*, ibid).

Previous work with the peptides and the question of permanence of amnesia has employed pretesting injections of ACTH-L to alleviate amnesia [23,24]. We have attempted to obtain similar effects using anisomycin to impair retention, but as yet we have not yet been successful in overcoming amnesia. A possible reason for the difference in results is that many studies compare trained treated and untreated animals. This comparison, while showing a loss of retention due to some amnestic treatment, does not necessarily imply that the loss of memory was complete or even substantial. In our studies, treated and untreated mice were compared against a performance criterion that represents the performance of naive mice. Treatments that can alleviate amnesia using pretesting injections may only be successful if the degree of memory loss is substantially less than complete. We recently reported that d-amphetamine reversed amnesia in anisomycin-injected mice which had long amnesic latencies in a passive avoidance task but had no effect on mice that originally had very short test latencies [14]. Presumably the amnesia was more severe in the case of those mice with very short test latencies and increased arousal was not an aid to recall. In addition, amphetamine had the same effect of improving recall in poorly trained saline-injected mice. Thus anti-amnestic effects are not necessarily restricted to animals receiving amnestic treatments. It might be said that anti-amnestic treatments have the ability to improve the recall of stored memories that are poorly stored either because of drug interference with consolidation or because of weak training as in the case of poorly trained undrugged animals.

Biochemical Effects of ACTH Peptides

While it is generally stated that ACTH and selected analogues stimulate protein synthesis, the clearest evidence for this stimulation comes from studies of hypophysectomized animals. In this system, either single or chronic injections have been shown to produce large increases in protein synthesis [20, 25, 26]. It should be noted that protein synthesis is depressed in these animals, and the effect of the peptides has been to restore protein synthesis to a more normal value. Another commonly used system to investigate the effects of ACTH analogues has been tissue slices of brain derived from both normal and operated animals. Here also stimulation of protein synthesis to a normal value was observed in the operated animal by in vitro addition of ACTH [22]. A surprising paucity of data appears to exist on the acute effects of ACTH and its analogues on cerebral protein synthesis. Rees *et al.* [21] have recently shown that ACTH peptides administered intracerebrally causes a slight stimulation of cerebral protein synthesis within 90 min after administration. Dunn *et al.* [8] have reported that a small but significant increased incorporation of lysine into brain protein within 15–25 min after administration of ACTH-L. Our studies are in general agreement with those of Rees *et al.* [21] in that any effects we found were small. It is clear that further research needs to be done to determine the effects of ACTH derivatives on protein synthesis at specific sites (micro effects).

In our experiments ACTH peptides had the ability to modify memory processing. ACTH-L facilitated memory processing and ACTH-D impaired processing. The effects were time-dependent and were observed in both passive and active avoidance tests. However, until more is known of the mechanics of action of these ACTH peptides, their role in memory processing will be uncertain.

ACKNOWLEDGEMENTS

We wish to express our appreciation to N. Belcher of Pfizer Pharmaceuticals for their generous gift of anisomycin, to Dr. H. Van Riezen of Organon International, Science development group, Holland for all ACTH peptides, and to Gary E. Smith and Daniel Vidal for skilled assistance for the behavioral experiments. The behavioral research was supported by NIMH grant NH 26608-01 to M.E. Jarvik, M.D., and the biochemical research by the U. S. Energy Research and Development Administration through the Laboratory of Chemical Biodynamics, Lawrence Berkeley Laboratory.

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[13]. In pathological conditions the regulation of cerebral blood circulation may be markedly abnormal [13, 14, 15].
4. Within certain limits cerebral blood flow is independent of changes of cardiac output, but in case of significant changes of cardiac output cerebral blood flow also changes; upon marked increase of cardiac output it slightly increases, upon marked decrease the volume of cerebral blood flow also decreases [16]. However, the cerebral fraction of cardiac output only increases considerably when vascular resistance of cerebral vessels is significantly reduced.

Thus improvement of the cerebral circulation by the use of drugs is a rather difficult and complex task, owing to the above described peculiar mechanisms of cerebral circulation. Increase of cerebral blood flow and O_2 supply may be expected only of vasodilators which do not induce systemic vasodilation, do not reduce blood pressure suddenly and significantly, and reduce cerebral vascular resistance in the first place (the type referred to as elective cerebral vasodilators). Upon the administration of Cavinton arterial mean pressure is reduced but slightly, while cerebral blood flow is slightly increased. The effect of Cavinton on vascular resistance asserts itself favourably in cerebral vascular areas in particular, decrease of systemic vascular resistance being much less marked than that of cerebral vascular resistance. On Cavinton the cerebral fraction of cardiac output significantly increases, evidencing the electively reducing effect of the drug on cerebral vascular resistance. According to the results of informative tests, cerebral metabolism did not change considerably on Cavinton. The heart rate did not change either during the tests and arrhythmia did not occur. The results of our studies indicate that Cavinton belongs to the rather few drugs which exert a potent, favourable effect on the cerebral circulation. The effect of Cavinton on the cerebral circulation has two main features:
1. It strongly reduces cerebral vascular resistance, which is typically high in cerebral vascular diseases;
2. cerebral fraction of cardiac output is increased.

No marked effect is exerted on systemic circulation, blood pressure and total vascular resistance decreased very slightly on acute Cavinton effect.

Since the drug, far from increasing rather reduces effort of the heart, its effect may be assumed to be favourable in cerebral alterations associated with heart disease and hypertension.

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Rheoencephalographic and Psychological Studies with Ethyl Apovincaminate in Cerebral Vascular Insufficiency

By D. Hadjiev and S. Yancheva

Summary: *The effect of ethyl apovincaminate (RGH-4405, Cavinton®) on the rheoencephalogram and memory functions was studied in 50 patients with ischaemic disturbances of cerebral circulation. The drug was administered in a single i.v. dose of 10 mg and orally three times daily 5 mg for a month. Improvement of cerebral circulation was observed after i.v. and oral medication. Blood flow was most markedly increased in the gray matter. The effect on arterial pressure was negligible. Improvement of memorizing capacity evaluated by psychological tests was recorded after one month of Cavinton treatment, associated with alleviation or complete disappearance of symptoms. No side-effects attributable to the drug were observed. It is pointed out that Cavinton is indicated in the treatment of ischaemic disorders of the cerebral circulation, particularly in chronic insufficiency.*

Zusammenfassung: *Rheoenzephalographische und psychologische Untersuchung von Äthyl-apovincaminat bei zerebrovaskulärer Insuffizienz*

Die Autoren untersuchten die Wirkung des Äthyl-apovincaminat (RGH-4405, Cavinton®) auf die Rheoenzephalographie und das Erinnerungsvermögen an 50 Patienten mit zerebralen Durchblutungsstörungen. Das Präparat wurde i.v. in einer Dosis von 10 mg/die und oral 3×5 mg/die einen Monat lang verabreicht. Sowohl bei der oralen Medikation als auch bei i.v. Verabreichung konnte eine Verbesserung des zerebralen Kreislaufes wahrgenommen werden. Die Durchblutung besserte sich am auffallendsten im Bereich der grauen Substanz. Die Verbesserung des Erinnerungsvermögens wurde mit Hilfe von psychologischen Tests ausgewertet, die nach einem Monat Behandlung mit Cavinton angefertigt wurden. Die Auswertung ergab eine Herabsetzung der konstitutionalen Symptome oder deren vollkommenes Verschwinden. Nebenwirkungen, die dem Präparat zugeschrieben werden könnten, traten nicht auf. Nach Meinung der Autoren ist Cavinton zur Behandlung von ischaemischen Veränderungen, besonders von chronischen Insuffizienzen, geeignet.

1. Introduction

The effect of ethyl apovincaminate (RGH-4405, Cavinton*)*) on the cerebral circulation has been the subject of several studies by the use of different methods: venous isotope dilution method [1], Doppler's ultrasonic technique [2] and rheoencephalography [3].

After i.v. administration of the drug Solti observed a fall of cerebral vascular resistance and increase in the cerebral fraction of cardiac output.

Rheoencephalographic studies in a small number of cases have shown improvement, absence of changes, occasionally even deterioration of cerebral circulation after i.v. or oral administration of the drug [3].

In an attempt to gain knowledge about the effect of Cavinton on the cerebral circulation, we studied the effect of the compound on the rheoencephalogram and on some mental functions in patients with cerebral infarction and cerebral ischaemia developed secondary to atherosclerosis and hypertension.

2. Material and methods

Rheoencephalographic recordings were made with the Schuhfried two-channel rheograph. Fronto-mastoid rheoencephalograms, their first derivatives and standard electrocardiograms were registered prior to and 1, 5, 10, 15 and 20 min after slow i.v. infusion (in 3—5 min) of 10 mg of Cavinton dissolved in 10 ml of a 10% dextrose solution.

Recordings were taken with the patients in a lying position. Arterial pressure was recorded at the same intervals, and mean pressure was calculated by the Wiggers formula.

In patients receiving the drug orally three times daily for a month, rheoencephalographic recordings were taken prior to and after the course of treatment.

Qualitative assessment was followed by quantitative analysis of a number rheoencephalographic parameters: amplitude, anacrotic section of the curve, tangent of the angle of inclination of the whole curve (tgα), tangent of the angle of inclination of slow systolic filling (tgα_1) and the amplitude of the first derivative. We also calculated the $\frac{Fg}{Fw}$ ratio, which gives information on the correlation between blood flow in the gray and white matter [4]. The changes in all these parameters were analyzed by statistical methods.

In patients receiving the drug orally psychological examination was carried out prior to and after medication. We used the "10 words" memorizing test [5] and required 10 immediate reproductions and one after an hour. We also interpreted the results of Wechsler's fifth subtest for evaluating the immediate accoustic-verbal memory for figures in normal and reverse order.

The effects of i.v. Cavinton injection were assayed in 30 patients — 14 females and 16 males, 30 to 80 years of age (average 59 years) [6]. 15 patients had non-obstructive cerebral infarctions, 11 thromboembolic infarction and 4 chronic cerebral circulatory insufficiency. In 19 patients brain damage was localized in the left cerebral hemisphere, in 8 in the right hemisphere, and in 3 in the vertebro-basilar system. All patients were in a recovery period or had residual symptoms after the disturbance of cerebral circulation.

*) Manufacturer: Chemical Works of Gedeon Richter Ltd., Budapest (Hungary).

The results of oral Cavinton medication were analyzed in 20 patients — 6 females and 14 males from 39 to 85 years of age (average age 59 years). 15 patients in this group had chronic insufficiency of cerebral circulation, 5 had a history of non-obstructive cerebral infarctions in the carotid system with residual pyramidal, sensory, vestibular and cerebrasthenic syndromes. 12 of the patients with chronic cerebral circulatory insufficiency had headache and vertigo before Cavinton treatment.

3. Results

A follow-up of the rheoencephalographic parameters in the intervals following i.v. Cavinton medication is given in Table 1. The percent changes of the parameters of the left hemisphere are shown in Fig. 1.

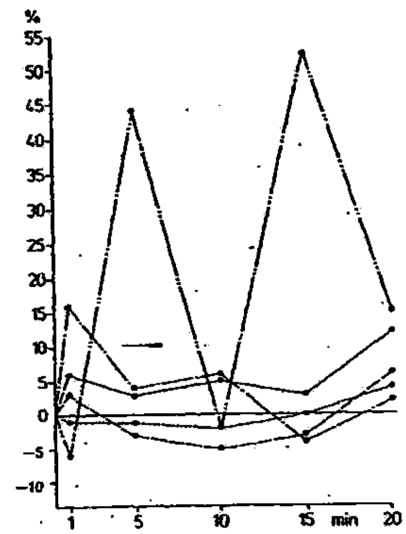

Fig. 1: Changes in rheoencephalograpic amplitude (——), anacrotic (———), tgα (————), tgα_1 (—————) and amplitude of the first derivative (·······) in the left hemisphere after i.v. injection of 10 mg Cavinton.

The rheoencephalographic amplitude, the anacrotic section of the curve and the amplitude of the first derivative show no essential changes, but tgα and tgα_1 increase significantly ($p<0.05$). Particularly distinct, though variable, are the changes in tgα_1, which reflect blood flow mainly in the small cerebral vessels.

The $\frac{Fg}{Fw}$ ratio for the left hemisphere increases from a pretreatment level of 1.84±1.02 to 2.19±0.88 after Cavinton injection. This change is associated with increased blood flow mainly in the gray matter.

The percent changes in the rheoencephalographic parameters of the right hemisphere following i.v. Cavinton are shown in Fig. 2.

The anacrotic section of the curve for the right hemisphere was not essentially altered, but the rheoencephalographic

Tab. 1: Changes in the basic rheoencephalographic parameters following i.v. injection of 10 mg Cavinton.

Parameters	Side	Prior to	1 min	5 min	10 min	15 min	20 min	p
Rheoencephalographic amplitude (ohms)	left right	0.078 0.075	0.083 0.081	0.081 0.086	0.082 0.079	0.081 0.083	0.087 0.092	>0.05 <0.001
Anacrote (sec)	left right	0.202 0.182	0.200 0.175	0.200 0.187	0.199 0.174	0.203 0.191	0.211 0.194	>0.05 >0.05
tg α	left right	0.82 0.90	0.95 0.99	0.85 0.96	0.87 1.02	0.79 0.97	0.84 0.97	<0.05 <0.001
tg α_1	left right	0.48 0.53	0.45 0.63	0.69 0.54	0.47 0.62	0.73 0.54	0.55 0.55	<0.01 <0.05
Amplitude of first derivative (mm)	left right	3.73 2.98	3.85 3.12	3.62 2.91	3.53 3.02	3.63 3.22	3.94 2.17	>0.05 <0.025

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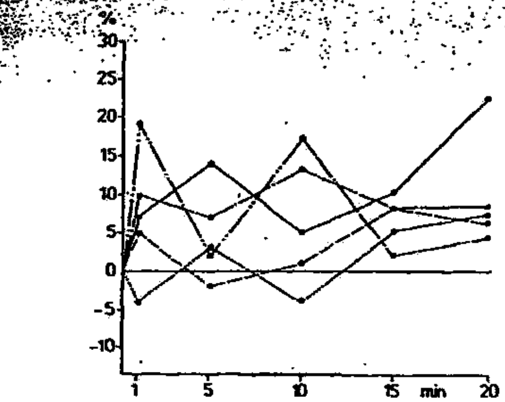

Fig. 2: Changes in rheoencephalographic amplitude (——), anacrote (– – –), tgα (———), tgα₁ (————) and amplitude of the first derivative (·······) in the right hemisphere after i.v. injection of 10 mg Cavinton.

amplitude, *tgα*, *tgα₁* and the amplitude of the first derivative significantly increased ($p<0.001$—$p<0.025$).

The changes in the rheoencephalographic parameters of the right hemisphere are more conspicuous and more stable than those of the left hemisphere.

The $\frac{Fg}{Fw}$ ratio increases, though insignificantly, from 1.63 ± 1.18 to 1.71 ± 0.96.

The rheoencephalographic changes after i.v. Cavinton may well be illustrated by some rheoencephalograms (Fig. 3).

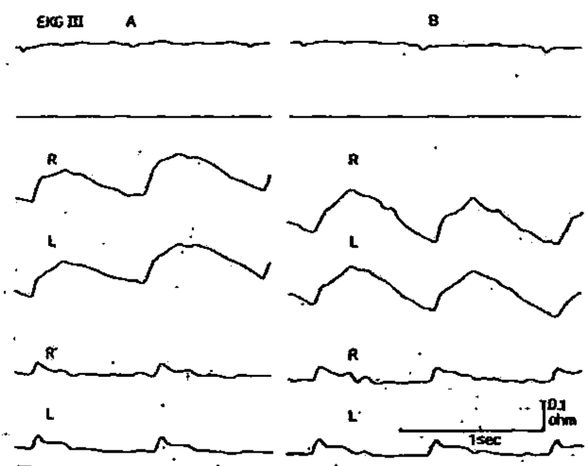

Fig. 3: MLT, male aged 68 years. Cerebral atherosclerosis. Thrombosis of left internal carotid artery. Right-side central hemiparesis. Fronto-mastoid rheoencephalograms and their first derivatives prior to (A) and after (B) i.v. injection of 10 mg Cavinton.

The systolic 156 ± 20 mm Hg, mean 111 ± 13 mm Hg and diastolic 89 ± 13 mm Hg blood pressure values showed minor changes after Cavinton medication. The only observation was a negligible, transient decrease in systole and mean pressures, most conspicuous in the 15th min (by 6 and 5 mm Hg, respectively).

The few patients reported on a sensation of warmth during Cavinton injection. No other side reactions were observed.

Improvement of symptoms was noted in patients receiving oral Cavinton: headache and vertigo diminished or entirely disappeared. All patients reported on being refreshed. Relief of pyramidal symptoms was observed in five patients. Repeated rheoencephalographic examination after one month in this group showed a tendency to improvement of cerebral hemodynamics: the peak of the rheoencephalogram became sharper, the catacrote details became more distinct, amplitude and *tgα* were increased.

The changes in the amplitude, anacrote and *tgα* of the right hemisphere are shown in Fig. 4. Analogous changes were observed in these same parameters for the left hemisphere.

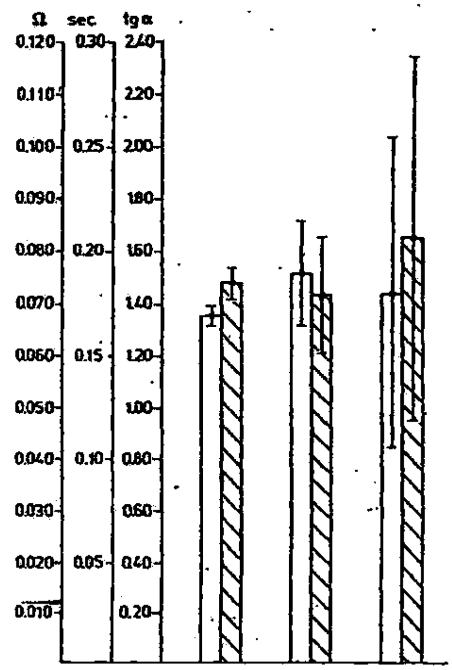

Fig. 4: Changes in rheoencephalographic amplitude, anacrote and tgα in the right hemisphere after (shaded columns) one month of Cavinton medication.

There was no change in systolic (141 ± 25 mm Hg), mean (104 ± 12 mm Hg) and diastolic (85 ± 7 mm Hg) blood pressure values in this group.

After Cavinton treatment for one month the "10 words" test showed essential improvement of memory functions. The changes observed are summarized in Fig. 5. In the "after one hour" test the number of reproduced words rose from 5.58 ± 3.06 to 6.23 ± 3.02 ($p>0.05$).

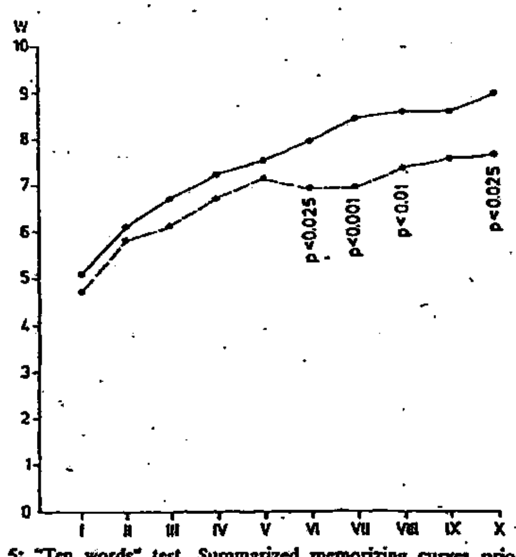

Fig. 5: "Ten words" test. Summarized memorizing curves prior to (– – –) and after (——) one month of Cavinton medication. Abscissa: number of experiments; W: Number of words memorized.

Wechsler's fifth subtest showed minor deviations. After treatment the number of figures repeated in normal order rose from 5.64 ± 0.99 to 5.76 ± 1.3; in reverse order from 3.64 ± 1.05 to 4.00 ± 1.00 ($p>0.05$).

In some patients memory functions were essentially improved, with accompanying favourable effect on cerebral hemodynamics. All this may be illustrated by the following observation: S.L.A., male, aged 64 years. Cerebral atherosclerosis. Cerebrasthenic syndrome. Complaints of headache, vertigo, forgetfulness and poor concentration. One month Cavinton medication was followed by essential improvement in the rheoencephalogram (Fig. 6). Moreover, memory functions evaluated by the "10 words" test were also favourably affected (Fig. 7). Before traetment the patient was able to reproduce only two words after one hour; at the end of the course of treatment the number of reproduced words rose to six. No side effects occurred after Cavinton treatment.

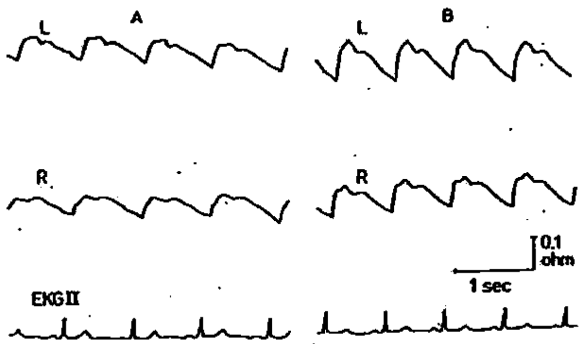

Fig. 6: S.L.A., male aged 64 years. Cerebral atherosclerosis. Cerebrasthenic syndrome. Fronto-mastoid rheoencephalograms prior to (A) and after (B) one month of Cavinton medication.

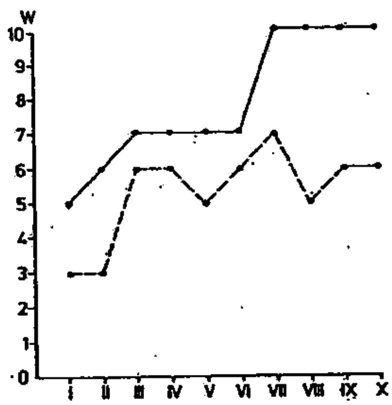

Fig. 7: Same patient. Memorizing curves prior to (– – –) and after (——) one month of Cavinton medication Abscissa: number of experiments; W: number of words memorized.

4. Discussion

Our observations showed that i.v. injection of 10 mg Cavinton induced dilatation of the cerebral vessels manifested by increase in rheoencephalographic amplitude, in tg$a$, tga_1 and the amplitude of the first derivative. These changes were less conspicuous and less stable in the left cerebral hemisphere in which the pathologic process, according to our observations, is localized more frequently. Obviously, the hemisphere affected by cerebral infarction has a lower vasodilator capacity.

The vasodilator action of Cavinton is exerted mainly on the arterioles. This is evidenced by increased blood flow in the phase of slow systolic filling. The rise in the $\frac{Fg}{Fw}$ ratio after i.v. Cavinton suggests more marked acceleration of blood flow in the gray than in the white matter. These rheoencephalographic changes correlate well with some polarographic investigations in dogs which showed a pronounced increase of blood flow in the gray matter [7].

In our studies after Cavinton injection we did not observe "steal" phenomenon with deterioration of cerebral circulation in the damaged and "intact" hemispheres.

The occasionally impaired cerebral circulation reported by Blatrix [3] after i.v. infusion of 30 mg Cavinton may obviously be attributed to the use of larger doses of the drug which could induce a more pronounced fall of blood pressure.

The favourable effect of i.v. Cavinton on the cerebral circulation persists until the end of the study period, in contrast to papaverine whose effect tends to wane from the 10th min following administration [8].

The absence of changes in arterial blood pressure following Cavinton injection suggests that the mechanism of action most probably rests on direct effect on vascular tone and not on the manifestation of preserved autoregulation, resulting from reduced intravascular pressure.

Favourable, though milder, rheoencephalographic changes were also observed after long-term oral intake of the drug. In these cases the improvement of cerebral haemodynamics was accompanied by favourable effect on memory disturbances evaluated by adequate psychological tests. Obviously, in cerebral ischaemia increased blood flow, predominantly in the gray matter, is followed by improvement of cerebral metabolism and function. It must be remarked here that this same mechanism is responsible for the disappearance of some neurological symptoms.

The cerebral vasodilator effect of Cavinton makes this drug suitable for the treatment of ischaemic disturbances of the cerebral circulation, especially of its chronic insufficiency. The therapeutic value of the drug is enhanced by the absence of side-effects and of changes in systemic arterial blood pressure, frequently induced by other vasodilator agents.

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Desglycinamide-9-Arginine-8-Vasopressin (DGAVP, Organon 5667) in Patients with Dementia[1]

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PEABODY, C. A., S. THIEMANN, R. PIGACHE, T. P. MILLER, P. A. BERGER, J. YESAVAGE AND J. R. TINKLENBERG. *Desglycinamide-9-arginine-8-vasopressin (DGAVP, Organon 5667) in patients with dementia.* NEUROBIOL AGING 6(2) 95–100, 1985.—Vasopressin peptides have been shown to facilitate learning and memory in both animals and humans; however, the effectiveness in humans is controversial. In a double blind parallel group study, 17 demented subjects (either Alzheimer's or alcoholic) were given either desglycinamide-9-arginine-8-vasopressin (DGAVP) 92 μg intranasally TID or an identical placebo for 1 week after having received 1 week of placebo. To our knowledge, this is the first report of DGAVP being used in subjects with dementia. The DGAVP group had a statistically significant improvement on the Buschke list learning of low imagery words. However, for various reasons discussed in the paper, we feel this finding needs to be replicated before any definite conclusions can be drawn. Since there were no other appreciable behavioral effects of this DGAVP regimen, our results should be considered negative. There was no evidence of any DGAVP-related adverse effects, except for possible weight gain.

Human studies Vasopressin DGAVP Memory Dementia

VASOPRESSIN (VP) peptides have been shown in laboratory animals to facilitate learning and memory in a variety of experimental conditions (for review see [7,32]). These positive findings provide a rationale for testing VP peptides in different human populations.

Patients with no evidence of dementia have been given clinical trials of VP peptides. In one study [22], 23 men between the ages of 50 and 65 who were hospitalized for minor pulmonary or gastrointestinal disorders but not screened for cognitive deficits, were randomly assigned to lysine vasopressin (LVP) or placebo treatment. The 12 patients who received 16 IU of LVP per day for 3 days performed better on attention and memory tasks. In an NIMH study four women between the ages of 41 and 52 with endogenous mood disturbances and cognitive impairment were treated with 60 to 160 μg of 1-desamino-8-D-arginine vasopressin (DDAVP) in a double blind, placebo-DDAVP-placebo design [12]. The treatment lasted 3–7 weeks. Three of the four subjects demonstrated significant enhancement during the DDAVP treatment in verbal learning performance, which returned to baseline levels by 6 weeks after the end of treatment.

Recently several groups have investigated vasopressin peptides in the treatment of dementia. In one study utilizing a randomized double blind cross-over design, 7 subjects with a diagnosis of early progressive idiopathic dementia were given DDAVP in doses of 30–60 μg/day [37]. Performance on a task involving free association of words in response to a letter or a category improved with the drug. In another study, 16 IU of LVP qd was used in a double blind placebo controlled parallel group design involving 17 Alzheimer subjects, 9 of which were in the active group [4, 8, 30]. Subjects receiving LVP showed a small improvement on a reaction time task, but did not improve in learning and memory tasks. The authors conclude that LVP did not significantly improve cognitive function. In a third study, using a double blind crossover design with 20 Alzheimer subjects, 10 IU of LVP bid was given for one week [9]. Small but statistically significant improvement was found on memory tasks involving fa-

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cial recognition and paired associate learning. A selective reminding task and a reaction time task did not show a significant drug effect, but there was a trend toward improvement on the reaction time task. On a self-rating mood factor scale, one factor labelled "carefree" showed a small but significant increase with LVP. This was interpretated as a possible stimulant effect.

Vasopressin peptides have also been used to treat cognitive impairments secondary to closed head trauma. LVP in doses of 11 to 30 IU per day improved both memory and mood in 3 patients with post-traumatic memory loss [26]. However, rigorous systematic assessments were not reported. Desglycinamide arginine vasopressin (DGAVP) has also been tried in post-traumatic amnesia. This peptide retains the behavioral effects of arginine vasopressin (AVP), the naturally occurring posterior pituitary hormone in man, but is devoid of the pressor or antidiuretic activity of AVP [6]. DGAVP in doses of 260 μg/day did not improve post-traumatic memory loss [17], perhaps because the patients were too impaired. The Utrecht group also had negative results in 6 patients with amnesia from severe trauma [19].

The purpose of the present study was to determine the effects of DGAVP on cognition and mood in subjects with dementia, either primary degenerative dementia (PPD, Alzheimer's disease, progressive idiopathic dementia) or dementia associated with alcoholism. It was the first systematic clinical trial of DGAVP in these disorders.

METHOD

Subjects

Seventeen subjects participated. Nine satisfied the Diagnostic and Statistical Manual (DSM-III) criteria for Primary Degenerative Dementia (PDD, Alzheimer's Disease) and also met the following criteria: insidious onset before the age of 65 with memory impairment as the initial feature, progressive dementia without stepwise deterioration for at least two years before this study, a Hachinski score [15] of less than 4, and no evidence of past or present alcoholism by DSM-III criteria. The other eight subjects satisfied the DSM-III requirements for Dementia Associated with Alcoholism, had at least one hospitalization for alcoholism, and had an Alcohol Addiction (ALCADD) score [23] of greater than 35. Additional exclusion criteria used for both groups were: no evidence of other causes of dementia, i.e., no cerebral vascular symptoms or signs, hypertension (150/95) or other cardiovascular disease, drug abuse, head trauma, epilepsy, or mental retardation; no localizing signs on EEG, CT or neurological exam; no abnormalities on chest X-ray and laboratory exams including B12, T3, T4, and folic acid; and no indication of significant depression or other psychiatric disturbances on two independent exams, with the exception of one patient with PDD who was depressed in addition to his dementia.

All subjects were mildly to moderately demented, were living at home, and in otherwise good physical health. They did not become disoriented in familiar settings and showed no marked personality deterioration. Most of these subjects had been employed in a position that required either a postgraduate degree or executive level management skills. However, none were working at the time of the study. Demographic and baseline clinical data for the treatment and control groups are shown in Table 1.

The study was approved by the Stanford Human Subjects Committee and was conducted at the Palo Alto Veterans Administration-Stanford–Mental Health Clinical Research Center.

TABLE 1

MEANS AND STANDARD DEVIATION (S.D.) ON THE DEMOGRAPHIC DATA AND INITIAL SCREENING DATA FOR THE ACTIVE GROUP (n=9; PDD=5) AND CONTROL GROUP (n=8; PDD=4)

	Active		Control	
Variable	Mean	S.D.	Mean	S.D.
Age	58.6	13.2	56.8	5.5
Education	15.3	3.1	14.8	2.3
Hamilton Depression	6.6	0.6	7.4	5.4
SCAG	35	3.3	38	2.5
Total Recall	8.1	3.3	7.5	2.8

There were no significant differences between groups on any variable using a two sample t-test.

Design

This is a report of two separate double-blind studies which were almost identical in design and have been combined for the purpose of analysis. In the first study there were 6 subjects. The active and control groups each consisted of 1 PDD and 2 alcohol subjects. In the second study there were 11 subjects. The active group consisted of 4 PDD and 2 alcohol subjects. The control group consisted of 3 Alzheimer and 2 alcohol subjects. In both studies after familiarization with the tasks and a baseline assessment, patients were randomly assigned to one of two parallel treatment groups. Each treatment group received placebo DGAVP spray intranasally (IN) three times a day (t.i.d.) for 5 days. Then the control group continued to receive placebo treatment for 5 more days, whereas the active group received DGAVP 92 μg IN, t.i.d. (about 202 μg/day free peptide) for the same period. Testing was done at 10 a.m. with the last dose being given at 7 a.m. In the first study there was one testing session at the end of both periods. In the second there were three testing sessions in each of the two periods. As the initial study progressed we became concerned that subject variability may be a problem so we increased the number of sessions in order to decrease the variability.

Measurements

Two verbal memory tasks were chosen that had been previously shown to be sensitive to drugs that may improve cognitive function in dementia. The first was a selective reminding verbal learning task [2,3], which has been found sensitive to vasopressin [36] and physostigmine [5,25]. The task consists of 12 words, 6 low imagery and 6 high imagery, balanced for frequency [31] and imagery [27] and given for 12 trials. The subject is reminded at the end of each trial of words not recalled on the previous trial. Two of the subscores include total recall (TR) and list learning (LL). TR is the highest number of words recalled on any trial. LL is the number of words recalled on the last trial that were also recalled on the previous trial. Subjects were initially selected

TABLE 2

PLACEBO AND DRUG MEANS FOR THE ACTIVE AND CONTROL GROUPS WITH S.E. (POOLED STANDARD ERROR OF THE DIFFERENCE) AND TWO SAMPLE t-TESTS COMPUTED ON THE RESPONSES

	Active		Control			
Measurement	Placebo	Drug	Placebo	Placebo	S.E.	p
Total Recall	8.9	9.0	7.5	8.0	0.10	0.002
List learning	4.8	5.7	3.2	2.8	1.5	n.s.
List learning low imagery	1.4	2.0	1.3	0.9	0.2	0.002
List learning high imagery	3.4	3.7	1.9	1.9	0.3	n.s.
20 word recall	2.8	4.8	4.3	4.1	3.9	n.s.
Hamilton Depression	7	6.2	4	4.1	1.3	n.s.
SCAG	36.3	34.5	32.8	33.8	1.0	0.02

on the basis of the TR score which had to be greater than 3 and could not reach 12 on 2 consecutive trials. The second cognitive task was a delayed free recall of 20 semantically related words [36]. The 10 minute delay was filled with a demanding distractor task.

Behavioral and mood assessments included the Sandoz Clinical Assessment Geriatric Scale (SCAGS), the Hamilton Depression (HamD), and the profile of Mood States (POMS).

Vital signs were recorded twice a day and weight once a day. Sodium, potassium, and plasma osmolality were measured twice a week. Complete physical and laboratory exams were performed at the beginning and the end of the study.

RESULTS

Each of the 2 studies described above was analyzed separately using a 2 sample t-test on the response. The response is defined as the drug (second treatment period) mean minus the placebo (first treatment period) mean. By suitably weighting the means and variances the 2 studies can be combined so that conclusions can be based on a larger sample. Three of the twenty-two measures were found to be statistically significant (Table 2). Although the control group did better on the total recall by one half of a word (or 7% improvement) and the active group did better on the SCAG by 2.8 points, both of these are of doubtful clinical significance. On the list learning-low imagery words (LL-LI), the active group increased their score by 0.6 of a word (or 43% improvement). The control group did worse by 0.4 of a word; thus the actual difference between the two groups was 1 word. It should be pointed out that the active group had higher scores overall, although the LL-LI scores were similar in the 2 groups. Five of the nine active subjects improved; three were alcoholics and two had PDD. As can be seen in Table 3 one of the difficulties in assessing the clinical significance with the PDD subjects is that the low imagery words were too difficult. As with normals [24], our subjects recalled significantly more high imagery than low imagery words (matched pairs t-test, $p<0.05$).

There were no indications of any serious side effects from DGAVP. There was a statistically significant change in weight (Table 4). The active group gained 0.9 of a pound and the placebo group lost 1 pound. There was no change in the blood pressure with treatment although baseline blood pressures were generally lower than one would expect from this age group.

DISCUSSION

A preliminary analysis suggests that DGAVP may improve learning of low imagery words in these subjects. However, based on the relatively small changes, the difference in baseline scores, and difficulty of the task for the PDD group, we do not feel that this conclusion is warranted. We are currently attempting to replicate this finding in a similar study using DDAVP instead of DGAVP. It is interesting to note that one group [28] has found that 10 g of choline in normal male subjects selectively enhanced storage and recall of low imagery words but not high imagery words, and that scopolamine has been found to abolish the normally found differences in recall between high and low imagery words. Taken together these findings underscore the importance of considering word imagability when analyzing drug effects on verbal learning. One should realize that word imagability may have more to do with word encodability than imagery, per se. Thus, drugs may differentially affect words that are normally difficult to encode.

In our study, as well as in the other studies discussed above in which vasopressin peptides were used in dementia, improvement in cognitive functioning has been either absent or minimal. The success of these peptides in rodents has been far greater. We have discussed elsewhere possible explanations for these discrepancies between positive animal findings and inconsistent human results [34]. One possible reason for animal-human differences may involve the efficiency with which exogenously administered vasopressin peptides penetrate any blood-brain barrier (BBB) and enter the central nervous system. Vasopressin peptides probably cross the barrier in rats [14,29]. It is unclear to what extent they enter the CNS in humans [16]. The efficiency of intranasal administration, which was used in this present study, is also a moot issue. In rhesus monkeys and in humans, radionuclide imaging of sodium pertechnetate 99 m sprayed intranasally has shown that the isotope is mainly deposited in the olfactory region in the monkey only, it

TABLE 3

PLACEBO AND DRUG MEANS FOR THE ALCOHOLICS (ALC) (n=8) AND PDDs (n=9)

		Active		Control	
Measurement	Diagnosis	Placebo	Drug	Placebo	Placebo
Total recall	Alc	11.3	11.5	7.8	8.2
Total recall	PDD	7.0	6.9	7.3	7.8
List learning	Alc	7.8	9.8	4.1	2.9
List learning	PDD	3.0	3.2	2.4	2.7
List learning low imagery	Alc	2.9	3.9	1.7	3.7
List learning low imagery	PDD	0.3	0.5	0.9	0.5
List learning high imagery	Alc	4.9	5.7	2.3	1.7
List learning high imagery	PDD	2.1	2.1	1.5	2.2
20 word recall	Alc	4.7	8.7	5.8	4.5
20 word recall	PDD	0.9	0.9	2.9	3.7
Hamilton	Alc	11	7.5	6.6	6.9
Hamilton	PDD	4.6	5.4	1.5	1.3
SCAG	Alc	29.3	26.6	36.0	37.1
SCAG	PDD	40.4	39.2	28.1	30.4

TABLE 4

BLOOD PRESSURE (BP), WEIGHT, SODIUM, AND PLASMA OSMOLALITY WITH t-TEST COMPUTED ON THE RESPONSES OF THE ACTIVE AND CONTROL GROUPS

	Active		Control		
Measurement	Placebo	Drug	Placebo	Placebo	p
Weight (lbs.)	164.1	165.0	151.5	150.5	0.04
BP-systolic	109.0	109.0	108.3	106.4	n.s.
BP-diastolic	73.1	74.5	69.8	69.9	n.s.
Serum Na	141.7	141.1	141.8	143.0	n.s.
Plasma osmolality	286.8	288.3	286.6	286.0	n.s.

migrated to the temporal region [13]. Colloidal gold was administered intranasally in the monkeys and particles were found in the olfactory neurons and the supporting cells and adjacent blood vessels. The authors conclude that administering drugs intranasally may be a convenient way to enter the central nervous system. When dogs were given intravenously iodine-125 labeled AVP, DGAVP, and DDAVP, radioactive substances measured in the CSF up to 50 minutes after administration amounted to less than 1.4% of the total given. Only DDAVP could be identified in the CSF unmetabolized. When AVP and DGAVP were given intranasally, there was no significant increase in the degree to which they entered the CSF [1]. As the authors point out it is possible that these peptides may metabolize in the brain and only their metabolites show up in the CSF. Another possibility is that these peptides may be transported by slow intraneuronal processes. In mouse olfactory cells intranasally administered albumin took 24 hours to reach the CNS [20]. Thus 50 minutes may be too short a time for either the peptides or their metabolites to enter the CSF.

Other possible reasons for our negative results with DGAVP may be incorrect dosage or inadequate period of administration. Since DGAVP had not been systematically used to treat dementia prior to our study, the dosage question is difficult to answer. We may have been using doses that were too low. Fink [10] reports that the acute administration of DGAVP in doses of 260 μg produced no changes in EEG in normal humans. Complete absences of EEG changes would be unusual for a behaviorally active drug, although not without precedent, especially for a CNS drug that is slow acting [33]. Alternatively, since we used only one dosage schedule for all subjects, we may have exceeded any behaviorally optimal dose range in some patients and did not reach it in others. The question of adequate length of administration is also difficult. If DGAVP is similar in its onset of action to DDAVP, one week might be sufficient since DDAVP has been reported to exert behavioral effects in humans within a few days [12,36].

Vasopressin may affect aspects of memory that have been termed extrinsic. Extrinsic aspects of memory refer to processes that can influence the development, maintenance, or expression of memory, but do not in themselves contain the representation of information as do the intrinsic components of memory. At a behavioral level, these extrinsic processes include selective attention, arousal, affect, general activation, and reinforcement. Several lines of evidence support the hypothesis that vasopressin may elevate affect or mood in certain individuals [11]. Because affect and cognition are intricately interrelated, enhancement of affect might improve cognitive performance. DGAVP did not discernibly change any parameters of mood or affect in our study, perhaps because our subjects were not particularly depressed (Hamilton baseline scores were relatively low as can be seen in

Table 1). Subjects who were more depressed might have shown a change in mood and cognitive function.

In summary, DGAVP treatment did not significantly improve verbal learning, mood or overall functioning in patients with early primary degenerative dementia or dementia associated with alcoholism. Of the several possible explanations for the discrepancies between our negative results and the positive animal findings, the most optimistic focuses on limitations in our study design. Low dosage schedule, too short an active treatment period, and perhaps excessively impaired patients are all possible explanations. Studies tha employ higher peptide doses for longer periods of time witl more appropriate patients are now being conducted. Result: from these trials should help to clarify any effects of vaso pressin peptides on human behavior.

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The Effects of ACTH- and Vasopressin-Analogues on CO_2-induced Retrograde Amnesia in Rats

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RIGTER, H., H. VAN RIEZEN AND D. DE WIED. *The effects of ACTH- and vasopressin-analogues on CO_2-induced retrograde amnesia in rats.* PHYSIOL. BEHAV. 13(3) 381–388, 1974. – Amnesia for a one-trial step-through passive avoidance response was induced in rats by application of CO_2 until respiratory arrest occurred. The ACTH-analogue $ACTH_{4-10}$ alleviated the amnesia when administered 1 hr prior to the retrieval test but not when given 1 hr prior to the acquisition trial. The behaviourally inert ACTH-analogue $ACTH_{11-24}$ appeared to have no effect on the amnesia. The vasopressin-analogue desglycinamide lysine vasopressin (DG-LVP) antagonized the amnesia when administered 1 hr prior to the acquisition trial or 1 hr prior to the test trial. The relevance of these date to present theories on amnesia is discussed.

$ACTH_{4-10}$ Amnesia Desglycinamide lysine vasopressin CO_2 Memory consolidation Memory retrieval

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FLEXNER and Flexner [6] reported that amnesia in mice induced by the intracerebral injection of puromycin was almost completely reversed when the animals were treated with an ACTH-preparation before or within 16 hr of the acquisition of an avoidance response. In a later investigation from the same laboratory [9], in which a chemically pure ACTH-preparation was used, no anti-amnesic effect could be detected; the originally observed anti-amnesic effect of ACTH was attributed to a vasopressin impurity. This assumption was validated by the finding that desglycinamine lysine vasopressin (DG-LVP) caused a reduction of the amnesia under the same circumstances as the crude ACTH-preparation used by Flexner and Flexner [6]. DG-LVP almost completely lacks the endocrine effects of vasopressin but exerts the same effects on learning behaviour in rats [25]. These results do not exclude an effect of ACTH on amnesia. Firstly, puromycin like other inhibitors of protein synthesis affects the function of the pituitary-adrenal axis [16], thereby possibly contaminating the effects of administration of exogeneous ACTH. Secondly, Flexner and Flexner [6] and Lande, Flexner and Flexner [9] administered ACTH prior to the amnesic treatment but not prior to the retrieval test. Furthermore, these investigators used ACTH, which apart from exerting an effect on the central nervous system also stimulates the production of corticosteroids from the adrenals. These corticosteroids may exert effects on behaviour which are opposite to those of ACTH [3,21]. Moreover, surgical removal of the adrenals, which causes increased ACTH release, results in a reduction of amnesia [5]. Although it is possible that adrenalectomy also causes an increased release of vasopressin [17], further studies on the possible anti-amnesic effect on ACTH seem justified. In the present investigation we compared the anti-amnesic effects of the ACTH-analogue $ACTH_{4-10}$ with the effects of DG-LVP. $ACTH_{4-10}$ lacks virtually all corticotrophic activities but possesses the same effects with respect to acquisition and extinction of avoidance behaviour as the parent hormone ACTH [22]. CO_2 was used as the amnesic agent. The efficacy of CO_2 as an amnesic agent compares favourably with that of the frequently used electroconvulsive shock [13,14].

METHOD

In each experiment 120 male rats of an inbred Wistar derived strain were used. They were obtained from TNO-Zeist, the Netherlands. The rats were 9–10 weeks of age

and weighed 230–260 g at the start of the experiments. The animals were housed 10 per cage (50 × 35 × 20 cm) with ad lib access to water and standard food pellets.

The step-through passive avoidance apparatus described by Ader, Weijnen and Moleman [1] was used. This consisted of a 40 × 40 × 40 cm Lucite chamber with black walls and a grid floor. The front wall was situated at the edge of a table. A 6 cm wide, 25 cm long runway protruded from the front wall over this edge. The runway was brightly lit by a 40 W lamp positioned 40 cm above it while the chamber was in darkness. When placed on the runway an animal could enter the chamber through a 6 × 6 cm opening in the front wall which could be closed by a hand-operated guillotine door. A scrambled foot shock could be delivered through the grid floor of the chamber. Shock was produced by a 500 V a.c. source through a variable resistance (0.5–5.5 MΩ). The resistance was adjusted to yield a shock intensity of 0.50 mA. The duration of the shock was controlled automatically and set for 3 sec.

The animals were randomly divided into 12 groups of 10. They were given 3 pretraining trials on Day 1 of the experiment. A pretraining trial consisted of placing the rat at the end of the runway while facing the open entrance. The latency of the animal to enter the chamber was recorded in tenths of a second. Upon entering the chamber with all 4 feet, the door was closed and the animal was left in the chamber for 10 sec. The interval between pretraining trials was approximately 2 hr. On Day 2 a single acquisition trial was run. It was identical to a pretraining trial except that at the end of it a foot shock was given to 8 groups of animals (FS groups). The remaining 4 groups were left undisturbed in the chamber for another 3 sec (No FS groups). Immediately on termination of the acquisition trial the rats were either subjected to amnesic treatment (CO_2 groups) or to sham amnesic treatment (No CO_2 groups). A 50 × 35 × 20 cm black plastic box with transparent cover was filled with CO_2 until oxygen measurements by means of a Teledyne Analytical Instruments Model 330 D Percent Oxygen Detector yielded zero readings. The rat was placed in the box until respiratory arrest occurred and then revived by artificial respiration. Following revival it was returned to its home cage. Pilot experiments indicated that it usually took 30–35 sec for respiratory arrest to occur. Therefore, rats receiving sham treatment were placed in an identical but air-filled box for 35 sec before being returned to their cages.

Twenty-four hr after acquisition a single test trial was run. When a rat did not enter the chamber within 300.0 sec, it was taken from the runway and a score of 300.0 sec was arbitrarily assigned to it.

The ACTH-analogues were dissolved in a phosphoric acid solution of pH 3.5. This solution was diluted with saline to a concentration of 100 μg/ml. It was neutralized to pH 7 with sodium bicarbonate before injection. DG-LVP was dissolved in a hydrochloric acid solution of pH 3.5. This solution was diluted with saline to a concentration of 10 μg/ml and subsequently neutralized with sodium bicarbonate to pH 7 prior to injection. Either 1 ml saline or 1 ml drug solution was injected s.c. 1 hr prior to the acquisition trial and/or the test trial. The experimental design is given in Table 1.

The results were analysed with the Yates test [26]. The test scores were divided into 3 classes: (1) latencies of 0–10.0 sec; (2) latencies of 10.1–299.9 sec; (3) scores of

TABLE 1

DESIGN OF EXPERIMENTS 1, 2 AND 3

Group	Foot Shock	CO_2	Treatment 1 hr prior to acquisition	Treatment 1 hr prior to test
No FS–No CO_2	–	–	saline	saline
No FS–No CO_2	–	–	saline	drug
No FS–CO_2	–	+	saline	saline
No FS–CO_2	–	+	saline	drug
FS–CO_2	+	+	saline	saline
FS–CO_2	+	+	drug	saline
FS–CO_2	+	+	saline	drug
FS–CO_2	+	+	drug	drug
FS–No CO_2	+	–	saline	saline
FS–No CO_2	+	–	drug	saline
FS–No CO_2	+	–	saline	drug
FS–No CO_2	+	–	drug	drug

FS = foot shock; No FS = no foot shock. CO_2 = CO_2-treatment; No CO_2 = no CO_2-treatment.

300.0 sec. Rats entering the chamber within 10.0 sec were considered to have no passive avoidance tendency. Rats entering within 10.1–299.9 sec were regarded to display an incomplete passive avoidance tendency. A refusal to enter within 300.0 sec was considered to be a complete avoidance response. In the analysis of the results the three classes received a statistical weighting of 0, 1 and 2 respectively.

EXPERIMENT 1

The effect of $ACTH_{4-10}$ on CO_2-induced amnesia for the one-trial passive avoidance response was studied. $ACTH_{4-10}$ was administered s.c. 1 hr prior to acquisition and/or retrieval test. The dose used was 100 μg/rat.

Results

Pretraining of the step-through response resulted in short latencies at the acquisition trial. Pretreatment with $ACTH_{4-10}$ did not affect the latencies at this trial (Table 2, part A). The No FS rats maintained short latencies at the test trial, irrespective of the pretreatment. No significant differences could be detected between No FS-No CO_2 and No FS-CO_2 groups (Table 2, part B). $ACTH_{4-10}$ did not affect passive avoidance behaviour in FS-No CO_2 groups, whether injected before the acquisition trial, the test trial, or before both trials (compared to the placebo FS-No CO_2 group: $z = 0.63$, 0 and 0.63, respectively) (Fig. 1).

TABLE 2

ACQUISITION AND TEST TRIAL STEP-THROUGH LATENCIES OF NON-SHOCKED GROUPS OF RATS FOLLOWING TREATMENT WITH $ACTH_{4-10}$

A				B			
Acquisition Trial				Test Trial			
group	number of rats	drug treatment	latency*† (sec)	group	number of rats	drug treatment	latency*† (sec)
pooled	80	saline	1.5 ± 0.2	No FS–No CO_2	10	saline	1.8 ± 0.2
				No FS–No CO_2	10	$ACTH_{4-10}$	1.7 ± 0.1
pooled	40	$ACTH_{4-10}$	1.6 ± 0.2	No FS–CO_2	10	saline	1.6 ± 0.2
				No FS–CO_2	10	$ACTH_{4-10}$	1.6 ± 0.2

*Mean ± standard error of the mean

†Differences between the groups in the column are not significant (two-tailed Mann-Whitney U test).

No FS–No CO_2: no foot shock, no CO_2-treatment

No FS–CO_2: no foot shock, CO_2-treatment

Saline: 1 ml saline s.c. 1 hr prior to trial

$ACTH_{4-10}$: 100 μg $ACTH_{4-10}$/rat s.c. 1 hr prior to trial

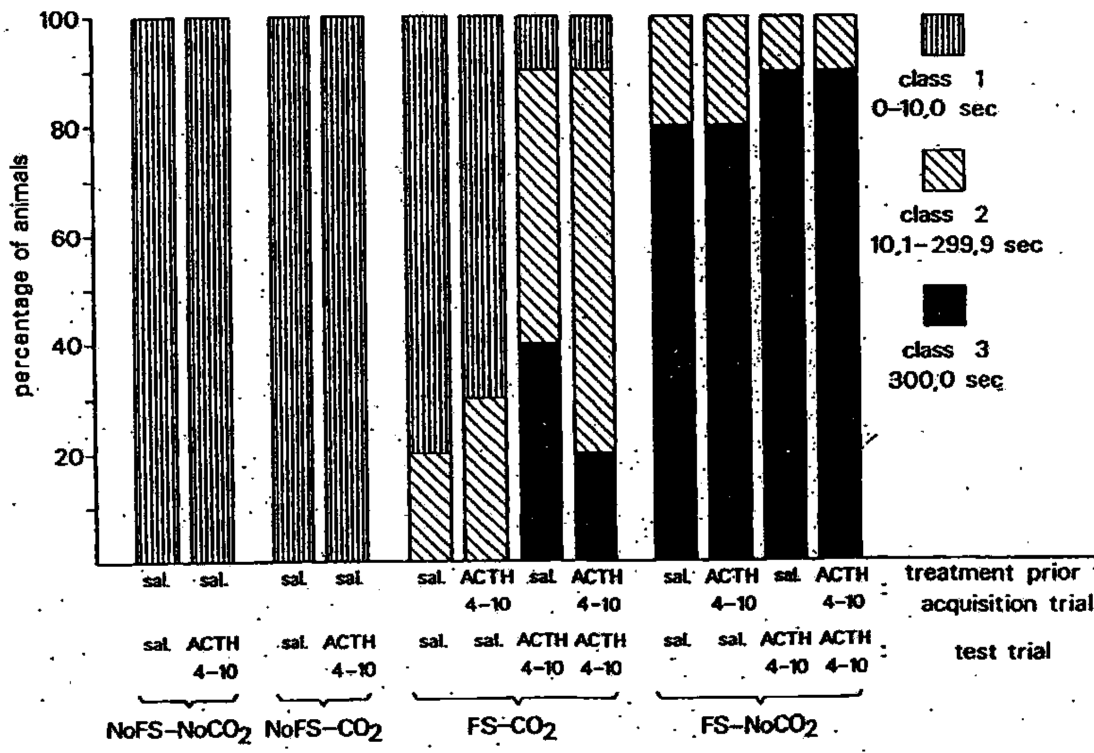

FIG. 1. The effect of $ACTH_{4-10}$ on CO_2-induced amnesia for a passive avoidance response. The figure presents the latencies at the test trial. The scores were divided into 3 classes: (1) 0–10.0 sec (no avoidance); (2) 10.1–299.9 sec (non-optimal avoidance); and (3) 300.0 sec (optimal avoidance). Saline: 1 ml saline/rat s.c. 1 hr prior to trial; $ACTH_{4-10}$: 100 μg $ACTH_{4-10}$/rat s.c. 1 hr prior to trial. FS: foot shock; No FS: no foot shock; CO_2: CO_2-treatment; No CO_2: No CO_2.

CO_2 was able to induce amnesia. This was apparent from the test scores of the placebo FS-CO_2 group: Only 2 out of 10 animals of this group had a latency longer than 10.0 sec. The scores of the other 8 rats were within the range of performance of the No FS groups.

Accordingly, the difference between the placebo FS-CO_2 and No FS-CO_2 groups was not significant (in both cases: $z = 1.49$, $p>0.05$). On the other hand, the difference between the placebo FS-CO_2 and the placebo FS-No CO_2 groups was significant ($z = 4.00$, $p<0.0001$) (Fig. 1).

When administered prior to the acquisition trial, $ACTH_{4\text{-}10}$ had no effect on the amnesia ($z = 0.52$, not significant). However, treatment with $ACTH_{4\text{-}10}$ 1 hr before the test trial resulted in a significant reduction of the amnesia. This was true for the group which received $ACTH_{4\text{-}10}$ before the test trial as well as for the group which received the peptide before both the acquisition and the test trial (compared to the placebo FS-CO_2 group: $z = 3.21$, $p<0.001$; and $z = 3.07$, $p<0.01$, respectively). However, the reduction of the amnesia due to pre-test treatment with $ACTH_{4\text{-}10}$ was not complete as comparisons with similarly treated FS-No CO_2 groups yielded significant differences ($z = 2.30$, $p<0.05$; and $z = 3.02$, $p<0.001$, for groups treated with $ACTH_{4\text{-}10}$ prior to the test trial and groups treated with $ACTH_{4\text{-}10}$ prior to both the acquisition and the test trial, respectively) (Fig. 1).

EXPERIMENT 2

Greven and de Wied [8] demonstrated that the effects of ACTH on acquisition and extinction of avoidance behaviour could be replicated by administration of various C terminal ACTH-analogues like $ACTH_{1\text{-}10}$ and $ACTH_{4\text{-}10}$. The sequence $ACTH_{11\text{-}24}$, however, was ineffective. The following experiment was designed to study whether $ACTH_{11\text{-}24}$ affected the CO_2-induced amnesia for the step-through passive avoidance response. $ACTH_{11\text{-}24}$ was injected in a dose of 100 μg/rat s.c.

Results

Administration of $ACTH_{11\text{-}24}$ did not alter the step-through latencies at the acquisition trial (Table 3, part A). Similarly, $ACTH_{11\text{-}24}$ did not affect the latencies of No FS rats at the test trial (Table 3, part B).

The peptide had no detectable influence on passive avoidance behaviour in FS-No CO_2 groups, whether injected before the acquisition trial, the test trial, or before both trials (compared to the placebo FS-No CO_2 group $z = 0.52$, 0 and 0, respectively) (Fig. 2).

The CO_2-treatment resulted in amnesia in the placebo FS-CO_2 group (compared to the placebo FS-No CO_2 group: $z = 4.00$; $p<0.0001$). The amnesia in this group was almost complete: the differences with the placebo No FS-No CO_2 and No FS-Co_2 groups were not significant (in both cases: $z = 1.49$).

Amnesia was unaffected by $ACTH_{11\text{-}24}$, whether injected before the acquisition trial, the test trial, or before both trials (compared to the placebo FS-CO_2 group: $z = 0.63$, 0.52 and 0.52, respectively, not significant; compared to the similarly treated FS-No CO_2 groups: $z = 4.02$, $p<0.0001$; $z = 3.88$, $p<0.0001$; and $z = 3.88$, $p<0.0001$, respectively). The $ACTH_{11\text{-}24}$-treated FS-CO_2 groups did not differ significantly from each other ($z<1.12$).

EXPERIMENT 3

DG-LVP has an anti-amnesic effect when administered prior to the acquisition of an avoidance response [9]. This is an effect which is qualitatively different to what was found in Experiment 1 for $ACTH_{4\text{-}10}$. This observation is in accordance with the general finding that vasopressin- and ACTH-analogues have different behavioural effects. Vasopressin exerts a long term effect on the extinction of active avoidance responses while the effect of $ACTH_{4\text{-}10}$ is of a short term nature [23,24]. Similarly, a single injection of DG-LVP results in a long term enhancement of passive

TABLE 3

ACQUISITION AND TEST TRIAL STEP-THROUGH LATENCIES OF NON-SHOCKED GROUPS OF RATS FOLLOWING TREATMENT WITH $ACTH_{11-24}$

A Acquisition Trial				B Test Trial			
group	number of rats	drug treatment	latency*† (sec)	group	number of rats	drug treatment	latency*† (sec)
pooled	80	saline	1.8 ± 0.2	No FS–No CO_2	10	saline	1.6 ± 0.1
				No FS–No CO_2	10	$ACTH_{11-24}$	1.7 ± 0.2
pooled	40	$ACTH_{11-24}$	1.7 ± 0.1	No FS–CO_2	10	saline	1.6 ± 0.2
				No FS–CO_2	10	$ACTH_{11-24}$	1.6 ± 0.2

*Mean ± standard error of the mean

†Differences between the groups in the column are not significant (two-tailed Mann-Whitney U test).

$ACTH_{11-24}$: 100 μg $ACTH_{11-24}$/rat s.c. 1 hr prior to trial.

See further the legend to Table 1.

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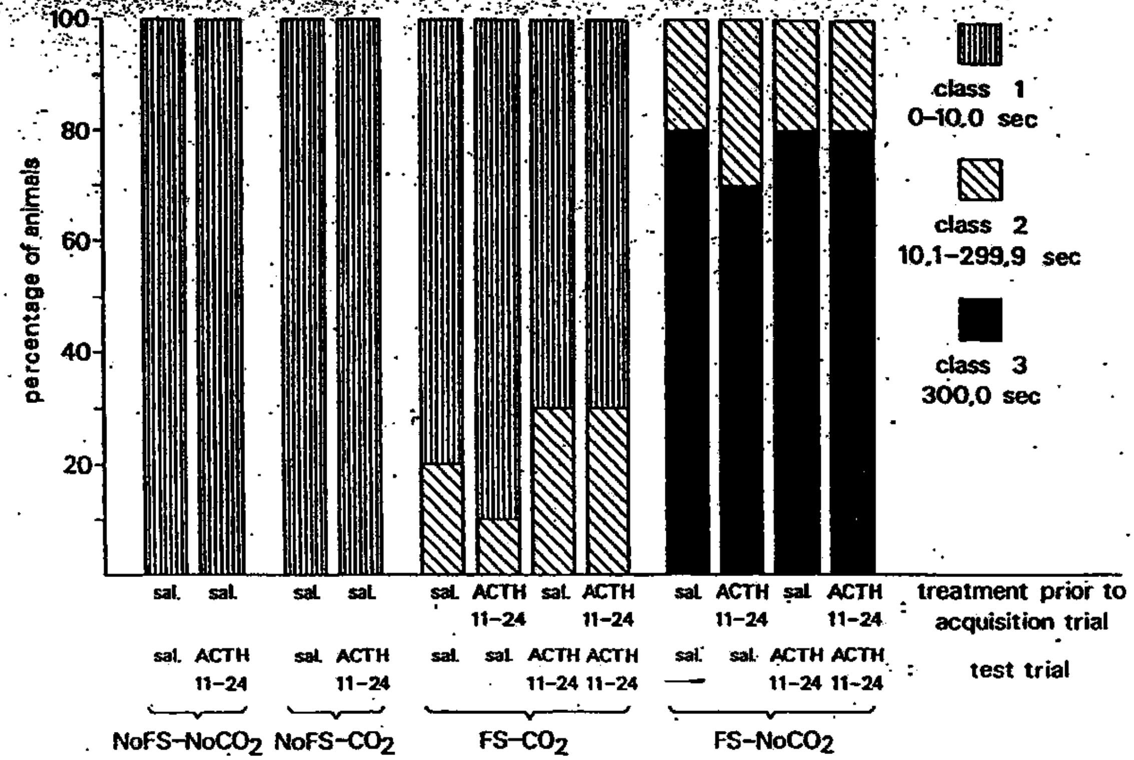

FIG. 2. The effect of $ACTH_{11-24}$ on CO_2-induced amnesia for a passive avoidance response. Saline: 1 ml saline/rat s.c. 1 hr prior to trial; $ACTH_{11-24}$: 100 µg $ACTH_{11-24}$/rat s.c. 1 hr prior to trial. See further the legend to Fig. 1.

TABLE 4

ACQUISITION AND TEST TRIAL LATENCIES OF NON-SHOCKED GROUPS OF RATS FOLLOWING TREATMENT WITH DESGLYCINAMIDE LYSINE VASOPRESSIN

A Acquisition Trial				B Test Trial			
group	number of rats	drug treatment	latency*† (sec)	group	number of rats	drug treatment	latency*† (sec)
pooled	80	saline	1.7 ± 0.1	No FS–NO CO_2	10	saline	1.8 ± 0.2
				No FS–No CO_2	10	DG–LVP	1.6 ± 0.2
pooled	40	DG–LVP	1.8 ± 0.1	No FS–CO_2	10	saline	1.7 ± 0.1
				No FS–CO_2	10	DG–LVP	1.7 ± 0.2

*Mean ± standard error of the mean
†Differences between the groups in the column are not significant (two-tailed Mann-Whitney U test).
DG–LVP: 10 µg desglycinamide lysine vasopressin/rat s.c. 1 hr prior to trial
See further the legend to Table 1.

avoidance behaviour whereas $ACTH_{4-10}$ only causes a temporary enhancement of this behaviour [19]. In the following experiment the effect of DG-LVP on CO_2-induced amnesia for the step-through passive avoidance response was studied. DG-LVP was administered in a dose of 10 µg/rat s.c.

Results

Pretreatment with DG-LVP had no effect on the step-through latencies during the acquisition trial (Table 4, part A). Similarly, DG-LVP did not affect the latencies of No FS animals during the test trial (Table 4, part B).

Passive avoidance of animals from FS-No CO_2 groups was unaffected by DG-LVP, whether injected before the acquisition trial, the test trial or before both trials (compared to the placebo FS-No CO_2 group: $z = 0.52$ in all cases; not significant).

CO_2 induced amnesia in the placebo FS-CO_2 group (compared to the placebo FS-No CO_2 group: $z = 4.13$, $p<0.0001$). Amnesia in this group was almost complete: the difference to the No FS-No CO_2 and No FS-CO_2 groups was not significant (in both cases: $z = 1.02$) (Fig. 3).

DG-LVP led to a reduction of amnesia when administered 1 hr prior to the acquisition trial (compared to the placebo FS-CO_2 group: $z = 3.04$, $p<0.01$). In addition, DG-LVP caused a reversal of amnesia when injected 1 hr prior to the test trial (compared to the placebo FS-CO_2 group: $z = 2.30$, $p<0.05$).

Administration of DG-LVP prior to both the acquisition and the test trial also resulted in a reduction of the amnesia (compared to the placebo FS-CO_2 group: $z = 2.65$, $p<0.01$). The DG-LVP-treated FS-CO_2 groups did not differ significantly from each other ($z<1.13$) (Fig. 3). The reduction of the amnesia in the DG-LVP-treated FS-CO_2 groups was not complete as comparisons with similarly treated FS-No CO_2 groups yielded significant differences ($z = 2.02$, $p<0.05$ for the group which received DG-LVP prior to the acquisition trial; $z = 2.99$, $p<0.01$ for the group which received DG-LVP prior to the test trial; and $z = 2.88$, $p<0.01$ for both the group which was treated with the peptide prior to both trials).

DISCUSSION

Amnesia can be antagonized by pharmacological treatments. The following compounds, for example, have been reported to reduce amnesia: amitriptyline [4]; Piracetam [7]; pentylenetetrazol [20] and strychnine [11]; α-methyl-para-tyrosine, propranolol and chlorpromazine [10]. This group of compounds is so pharmacologically heterogeneous that a uniform mechanism of action seems to be excluded. Most of these investigations offer insufficient guarantees that the anti-amnesic effect of these compounds has not been brought about in a non-specific way. For, in general, these investigations made use of only one type of behavioural task (avoidance tasks), and of only one type of amnesic agent (electroconvulsive shock).

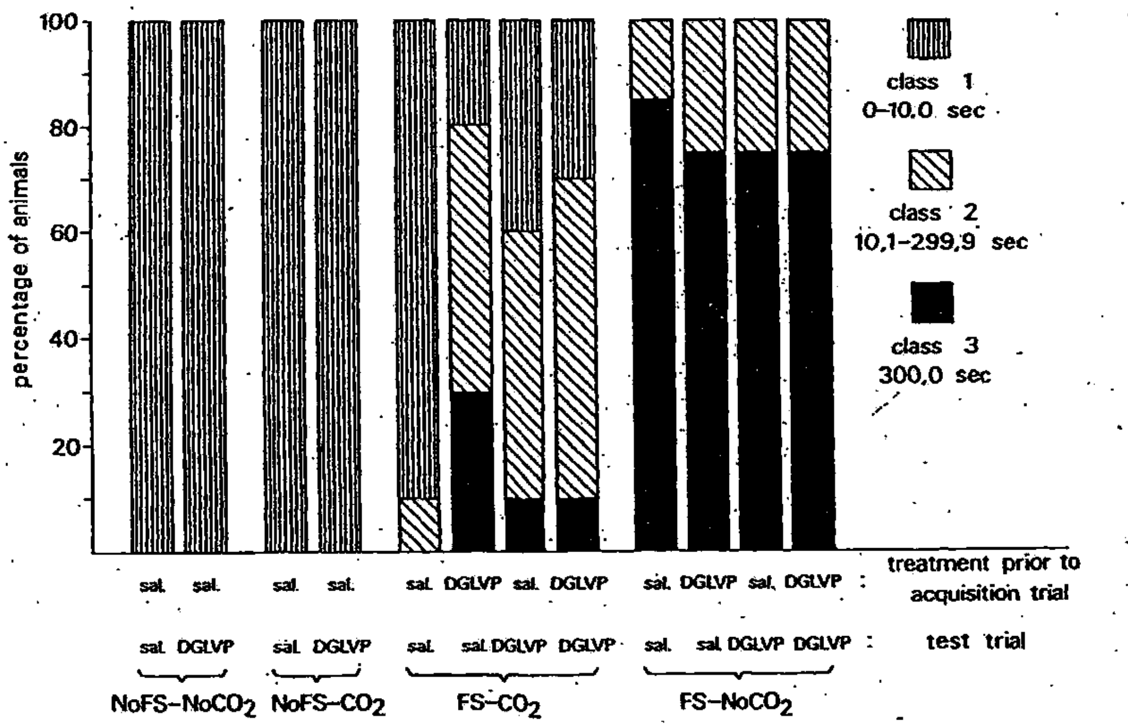

FIG. 3. The effect of desglycinamide lysine vasopressin on CO_2-induced amnesia for a passive avoidance response. Saline: 1 ml saline/rat s.c. 1 hr prior to trial; DGLVP: 10 µg desglycinamide lysine vasopressin/rat s.c. 1 hr prior to trial. See further the legend to Fig. 1.

Furthermore, due to the assumption that amnesia is based on a disruption of memory consolidation, in most of these studies the drug was only administered shortly before or after application of the amnesic agent. Recent theories, however, hold that amnesia is based on a disturbance of memory retrieval [12,18]. Accordingly, the possibility exists that the administration of a drug shortly before the retrieval test may lead to a reduction of amnesia.

In the present studies these objections were partially met by examining the effect of administration of a drug prior to the acquisition trial as well as the test trial on the CO_2-induced amnesia for a step-through passive avoidance response. Elsewhere, we report similar studies, in which we used a different amnesic agent and a different behavioural task [15].

In Experiment 1 it was found that the peptide $ACTH_{4-10}$ can alleviate the amnesia when administered 1 hr prior to the test trial. Administration of the peptide 1 hr prior to the acquisition trial was ineffective. It is improbable that the anti-amnesic effect of $ACTH_{4-10}$ is due to an influence on locomotor capacities of the experimental animals as $ACTH_{4-10}$ did not affect the step-through latencies of non-shocked animals. $ACTH_{4-10}$ did not affect the passive avoidance behaviour of FS-No CO_2 rats. However, in the present paradigm it was not possible to measure an increased tendency to avoid as most of the placebo FS rats made already a complete avoidance response.

The results of Experiment 1 suggest that $ACTH_{4-10}$ promotes the retrieval of memory items which are affected by the amnesic treatment. This effect can be explained in three ways: (1) $ACTH_{4-10}$ strengthens the passive avoidance tendency. Such an effect may be inferred from the work of Thompson and de Wied [19]. However, Rigter and van Riezen [15] demonstrated that $ACTH_{4-10}$ is also able to reverse the CO_2-induced amnesia for a thirst-motivated response. This result indicates that an increased tendency to avoid is not sufficient to explain the reduction of amnesia caused by $ACTH_{4-10}$. (2) $ACTH_{4-10}$ promotes the retrieval of weak memory items. This explanation does not limit itself to memory items concerned with passive avoidance. It is possible that some or part of the relevant memory item(s) survive the amnesic treatment and that $ACTH_{4-10}$ facilitates the retrieval of these item(s) at the test trial. In using this explanation, one can leave undecided whether amnesia is based on a disturbance of memory consolidation or on a disturbance of memory retrieval. This explanation is in keeping with previous findings: an improved retrieval may be responsible for the facilitation of acquisition and the delay of extinction of avoidance behaviour following treatment with $ACTH_{4-10}$ [8,22]. (3) $ACTH_{4-10}$ promotes the retrieval of memory by reversing the disturbance of retrieval induced by the amnesic treatment. Explanation 3 is not necessarily incompatible with Explanation 2. To test this possibility, it is necessary to develop a behavioural test which is able to discriminate between a disturbance of memory consolidation and a disturbance of memory retrieval.

The reversal of amnesia by treatment with $ACTH_{4-10}$ did not occur in all animals. It is improbable that this is due to an inadequate dose of the peptide as in another study it was found that a dose of 10 μg $ACTH_{4-10}$/rat already exerts an anti-amnesic effect and that this effect cannot be increased by augmenting the dose to 100 μg/rat [14]. Therefore, it can be assumed that 100 μg $ACTH_{4-10}$ is an adequate dose.

In contrast to $ACTH_{4-10}$, $ACTH_{11-24}$ did not influence the CO_2-induced amnesia for the passive avoidance response. This finding suggests that the anti-amnesic effect of ACTH can be traced to the same amino acid sequence (i.e., 4–10) which has been found effective in delaying the extinction of avoidance response [22].

In keeping with the results of Lande, Flexner and Flexner [9] it was found in Experiment 3 that DG-LVP caused a reduction of the CO_2-induced amnesia for the passive avoidance response when injected 1 hr prior to the acquisition trial. Moreover, it was demonstrated that DG-LVP exerts the same effect when administered 1 hr prior to the test trial. These findings indicate that DG-LVP has a qualitatively different effect to $ACTH_{4-10}$. Other investigations have led to the same conclusion [2,24].

The finding that DG-LVP has an anti-amnesic effect when administered prior to the acquisition trial suggests that this peptide is able to promote memory consolidation either by facilitating the consolidation process or by protecting memory consolidation from the adverse effects of the amnesic treatment. However, the possibility that administration of DG-LVP prior to acquisition influences later retrieval cannot be excluded. There is some evidence in favour of this hypothesis. The fact that DG-LVP has an anti-amnesic effect when injected prior to the test trial suggests that this compound promotes memory retrieval either by facilitating the retrieval process or by reversing a CO_2-induced disturbance of retrieval. The effect of preacquisition treatment with DG-LVP may be based on the same mechanism.

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Anti-Amnesic Effect of $ACTH_{4-10}$: Its Independence of the Nature of the Amnesic Agent and the Behavioral Test

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RIGTER, H. AND H. VAN RIEZEN. *Anti-amnesic effect of $ACTH_{4-10}$: Its independence of the nature of the amnesic agent and the behavioral test.* PHYSIOL. BEHAV. 14(5) 563–566, 1975. — It was found previously that $ACTH_{4-10}$ is able to alleviate the CO_2-induced retrograde amnesia for a step-through passive avoidance response when injected into rats 1 hr prior to the retrieval test. The present investigation was undertaken to establish whether $ACTH_{4-10}$ has a similar effect if a different amnesia agent and a different behavioral task are used. It appeared that electroconvulsive shock induced amnesia for a one-trial thirst-motivated response. This amnesia could be reduced by administration of $ACTH_{4-10}$ 1 hr prior to the retrieval test. Administration of $ACTH_{4-10}$ 1 hr prior to the acquisition was ineffective. It was concluded that $ACTH_{4-10}$ exerts a retrieval-promoting effect independent of the nature of the amnesic agent and the behavioral task.

$ACTH_{4-10}$ Retrograde amnesia CO_2 Electroconvulsive shock Retrieval One-trial appetite-motivated response

IT is well-established that retrograde amnesia can be antagonized by treatment with pituitary hormones or hormonal derivatives [5, 6, 8]. Quinton [6] reported that ACTH alleviated cycloheximide-induced amnesia in mice. It is uncertain whether this effect was brought about by ACTH itself, or by an ACTH-induced release of corticosteroids from the adrenal cortex. However, Rigter, van Riezen and de Wied [8] demonstrated that the ACTH-analogue, $ACTH_{4-10}$, which is virtually devoid of adrenocorticotrophic activity [15,17], partially reversed carbon dioxide (CO_2)-induced amnesia in rats. This suggests that the anti-amnesic effect of ACTH is not dependent on the adrenal cortex.

A detailed analysis of the anti-amnesic effect of $ACTH_{4-10}$ indicated that this peptide was able to antagonize CO_2-induced amnesia for a passive avoidance response when given 1 hr prior to the retrieval test but not when given 1 hr prior to the acquisition trial [8]. These results suggested that $ACTH_{4-10}$ promoted the retrieval of the avoidance response. The aim of the present investigation was to assess if $ACTH_{4-10}$ can affect amnesia for a different type of behavioral response, and whether the anti-amnesic effect can also be obtained when an amnesic agent other than CO_2 is used. Therefore, the effect of $ACTH_{4-10}$ on the electroconvulsive shock (ECS)-induced amnesia for an appetite-motivated response was studied.

METHOD

Animals

Sixteen groups of 10 male Wistar rats weighing 200–230 g were used. The animals were housed 8 per cage (50 x 3 x 20 cm). The light-dark cycle and the temperature of th animal room were controlled automatically. Light was o from 7:30 a.m. to 19:30 p.m. Temperature was set fc 21°C. The animals had ad lib access to standard food pelle but were maintained on a 23.5 hr water deprivatio schedule for the duration of the experiment.

Amnesic treatment

Electroconvulsive shock was used as the amnesic agen Two platinum needle electrodes were inserted into the sk at the innerside of the ears of the rat. The electrodes we connected with a double flexible wire to a S8 Gra Stimulator. The current intensity of the ECS was adjuste by means of a Grass Constant Current Unit. The EC consisted of a single pulse train with a frequency of 60 He and a duration of 500 msec. The pulse width was 10 ms and the intensity 30 mA. Rats receiving sham amnesic trea ment were attached to the electrodes but were n subjected to ECS.

Procedure

The experiment was run in 10 randomized blocks. Eac block contained 16 rats. The experiment was carried out a normally lit, sound-proof room. The rats were placed o at a time in the middle of a circular open field (55 c diameter, 50 cm high wall). The floor was covered wi sawdust. A grey perspex pillar was placed against the w of the open field. A 5 x 5 x 8 cm niche was made at t bottom of the pillar through which the spout of an emp

water bottle protruded. In the niche, a light beam and a photocell were located. When the rat explored the niche, the light beam was interrupted thereby activating a counter which recorded the duration of the interruption (4 counts/sec). Thus it was possible to record the total duration of niche explorations which a rat made during a trial.

The animals were placed in the open field 3 min a day during 5 consecutive days. On Day 6 the water bottle in the pillar was filled. Eight groups of rats (W group) were allowed to drink from the spout for 37.5 sec. Immediately on termination of the drinking period the rats were subjected to either amnesic treatment (W-ECS groups) or to sham amnesic treatment (W-NoECS groups). Eight other groups were not placed in the open field on Day 6. Instead, they were allowed to drink in a perspex cage for approximately 37.5 sec (NoW-groups). Four groups were subjected to ECS immediately on termination of the drinking period (NoW-ECS) whereas the other 4 groups received sham amnesic treatment (NoW-NoECS).

A 3 min retrieval test was given on Day 7. At this test, the water bottle in the pillar was empty. An increase in the total duration of the niche exploration on Day 7 compared to the score on Day 5 was considered to be a measure of retention. The absence of such an increase due to ECS-treatment was seen as a measure of amnesia.

Drug treatment. $ACTH_{4-10}$, dissolved in a phosphoric acid solution pH = 3.5, was diluted with saline and neutralized with sodium bicarbonate before injection. The vehicle, similarly diluted and neutralized, was used as placebo. Either 1 ml $ACTH_{4-10}$ (100 μg/rat) or 1 ml vehicle was injected sc 1 hr prior to the trials on Day 6 and 7 according to the schedule outlined in Table 1.

Statistical analysis. Previous experiments showed that the scatter of Day 7 scores differed between NoW, W-ECS and W-NoECS groups (Rigter, unpublished observations). Moreover, it appeared that the correlation between Day 7 and Day 5 scores also varied considerably between NoW, W-ECS and W-NoECS groups. Therefore, a single four-factor analysis was considered to be invalid. Instead, separate analyses (two- and three-factor randomised block analyses of covariance) were applied to the NoW, W-ECS and W-NoECS groups, respectively, to assess the signifi-

TABLE I

THE EFFECT OF $ACTH_{4-10}$ ON THE ECS-INDUCED AMNESIA FOR A THIRST-MOTIVATED RESPONSE

Group	Treatment on* Day 6	Treatment on* Day 7	Mean Exploration Score on† Day 5	Mean Exploration Score on† Day 7
NoW-NoECS	Placebo	Placebo	76.3 ± 8.5	56.2 ± 5.9‡
	$ACTH_{4-10}$	Placebo	70.9 ± 12.8	50.3 ± 9.2‡
	Placebo	$ACTH_{4-10}$	72.5 ± 10.1	42.9 ± 8.5‡
	$ACTH_{4-10}$	$ACTH_{4-10}$	56.0 ± 8.6	39.9 ± 7.0
NoW-ECS	Placebo	Placebo	62.4 ± 8.5	39.2 ± 5.7‡
	$ACTH_{4-10}$	Placebo	72.7 ± 12.7	46.5 ± 8.0
	Placebo	$ACTH_{4-10}$	51.5 ± 7.8	40.5 ± 8.1‡
	$ACTH_{4-10}$	$ACTH_{4-10}$	84.5 ± 7.8	61.1 ± 10.7‡
W-ECS	Placebo	Placebo	70.9 ± 7.3	80.8 ± 16.6
	$ACTH_{4-10}$	Placebo	51.6 ± 9.8	63.3 ± 15.0
	Placebo	$ACTH_{4-10}$	76.2 ± 14.1	149.5 ± 16.9§
	$ACTH_{4-10}$	$ACTH_{4-10}$	61.1 ± 10.8	119.0 ± 11.0§
W-NoECS	Placebo	Placebo	58.0 ± 10.1	123.0 ± 19.9§
	$ACTH_{4-10}$	Placebo	79.4 ± 8.8	129.5 ± 15.2§
	Placebo	$ACTH_{4-10}$	54.0 ± 9.6	176.1 ± 12.4§
	$ACTH_{4-10}$	$ACTH_{4-10}$	60.8 ± 8.5	129.1 ± 12.5§

W-groups were subjected to the acquisition trial whereas NoW groups were not. ECS: groups receiving amnesic treatment; NoECS: sham treatment. Ten rats per group.

*Either 1 ml vehicle solution or 100 μg $ACTH_{4-10}$/rat was sc injected 1 hr prior to acquisition (Day 6) or retrieval (Day 7).

†Mean number of photocell counts (4/sec) ± standard error of the mean.

‡Significantly decreased exploration on Day 7 compared to Day 5 ($p<0.05$; two-tailed randomization test).

§Significantly increased exploration on Day 7 compared to Day 5 ($p<0.05$; two-tailed randomization test).

TABLE 2

DIFFERENCES IN EXPLORATION BETWEEN GROUPS OF RATS DURING THE RETRIEVAL TEST

Condition	Administration of $ACTH_{4-10}$ Prior to:	Increase of Exploration*	95% Confidence Interval
W-ECS	Acquisition	−24	−57, +9
	Retrieval	+62†	+29, +95
W-NoECS	Acquisition	−20	−54, +13
	Retrieval	+26	−7, +60

Within each condition 4 groups of 10 rats were used: 1. (pre-acquisition) placebo-(pre-retrieval) placebo; 2. placebo-$ACTH_{4-10}$; 3. $ACTH_{4-10}$-placebo; and 4. $ACTH_{4-10}$-$ACTH_{4-10}$. In order to assess the effect of pre-acquisition administration of $ACTH_{4-10}$, Groups 3 + 4 were compared with Groups 1 + 2. The effect of pre-retrieval administration of $ACTH_{4-10}$ was determined by comparing Groups 2 + 4 with Groups 1 + 3.

*Difference between the mean exploration score (number of photocell counts) of the combined $ACTH_{4-10}$-treated groups and combined placebo-treated groups.

†$p<0.05$

cance of group differences at Day 7. Day 5–Day 7 comparisons were made by means of the randomization test for matched pairs [13].

RESULTS

The mean Day 5 and 7 exploration scores are given in Table 1. The Day 7 exploration scores of 3 NoW-NoECS and 3 NoW-ECS groups were significantly lower than the Day 5 scores (randomization test; Table 1). In comparison to their Day 5 scores, there was no significant change in the Day 7 exploration scores of the W-ECS groups which received the vehicle solution prior to the retrieval test on Day 7; however, the W-ECS groups which were treated with $ACTH_{4-10}$ prior to the retrieval test had increased exploration scores. Similarly, all W-NoECS groups showed increased exploration on Day 7 (Table 1).

The Day 7 scores were analysed in three parts by means of separate analyses of covariance using the Day 5 score as covariate. In the first analysis, the 8 NoW groups were compared. In spite of the corrections made for the significant contributions associated with the Day 5 scores, neither ECS, nor administration of $ACTH_{4-10}$ (either prior to the acquisition or prior to retrieval) yielded significant differences between groups. Moreover, the interactions between these three factors were not significant.

The second analysis of Day 7 scores concerned the 4 W-NoECS groups. Administration of $ACTH_{4-10}$ either prior to acquisition or prior to retrieval did not affect performance (Table 2). The interaction between these two factors was not significant.

The third analysis pertained to the 4 W-ECS groups. Administration of $ACTH_{4-10}$ prior to acquisition did not influence retrieval. However, $ACTH_{4-10}$ prior to retrieval resulted in significantly increased exploration scores (Table 2). There was no significant interaction between preacquisition and preretrieval treatment with the peptide.

DISCUSSION

Thirsty W-NoECS rats showed increased exploration of a niche in which they had found water 24 hr earlier; in contrast, NoW-NoECS and NoW-ECS rats which had no previous experience of water in the niche showed decreased exploration. Therefore, an increase of exploration can be considered as a measure of retention. When acquisition was followed by treatment with ECS, the increase of exploration did not occur: it is concluded that ECS induced amnesia. The ECS-induced amnesia could be reversed by administration of $ACTH_{4-10}$ 1 hr prior to retrieval test. Administration of $ACTH_{4-10}$ 1 hr prior to acquisition was ineffective. These results can not be attributed to an effect of the peptide on exploratory behavior or thirst. Weijnen and Slangen [14] reported that $ACTH_{4-10}$ did not influence exploration. In keeping with this report, it was found in the present investigation that the exploration scores of NoW groups which were treated with the peptide did not differ from those of the placebo-treated NoW groups. It is improbable that $ACTH_{4-10}$ affected thirst since neither ACTH nor $ACTH_{4-10}$ have an effect on water intake ([15]; Rijk and van Riezen, unpublished results).

The results are in accord with the previous finding that $ACTH_{4-10}$ is able to reduce CO_2-induced amnesia for a passive avoidance response or for the appetite-motivated response as used in the present study when injected prior to the retrieval test but not when administered prior to acquisition ([8]; Rigter, unpublished results). In addition, Quinton [6] reported that ACTH given prior to retrieval attenuated cycloheximide-induced amnesia in mice. It is probable that CO_2, ECS and cycloheximide exert their amnesic effect via different mechanisms [7]. Since $ACTH_{4-10}$ (or ACTH) was able to reduce amnesia induced by all three agents, it is concluded that the anti-amnesic effect of $ACTH_{4-10}$ can not be explained by an interaction with specific characteristics of an amnesic treatment. Furthermore, the finding that $ACTH_{4-10}$ reduced amnesia for different behavioral tasks indicates that the anti-amnesic effect of this peptide is not due to an interaction with specific task parameters. These conclusions strengthen our hypothesis [8] that $ACTH_{4-10}$ promotes the retrieval of memory items which are affected by amnesic treatments.

Two explanations can be offered: (1) $ACTH_{4\text{-}10}$ promotes the retrieval of (weak) memory items. It is possible that some or part of the relevant memory item(s) survive the amnesic treatment and that $ACTH_{4\text{-}10}$ facilitates the retrieval of these item(s) at the test trial. (2) $ACTH_{4\text{-}10}$ promotes the retrieval of memory by reversing the disturbance of retrieval induced by the amnesic treatment. The present results favour the second explanation as it was demonstrated that $ACTH_{4\text{-}10}$ did not change retrieval of the appetite-motivated response in the W-NoECS groups.

A variety of behavioral effects of ACTH, $ACTH_{4\text{-}10}$ and MSH (which has the same 4-10 amino acid sequence as ACTH) have been described. These compounds delay the extinction of fear-motivated and appetite-motivated responses [1, 2, 9] and facilitate passive avoidance behavior [2, 3, 10]. Several hypotheses have been put forward to account for these behavioral effects: increased motivation [12]; increased attention [11]; and increased motivational value of environmental stimuli [16]. These hypotheses are not necessarily incompatible with our statement that $ACTH_{4\text{-}10}$ promotes retrieval of memory. Memory retrieval probably involves attentional components. Therefore, the $ACTH_{4\text{-}10}$-induced improvement of retrieval may be due either to a general increase of attention (to motivationally relevant stimuli) or to the reversal of a specific attentional deficit.

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Neurotransmitter Precursor Amino Acids in the Treatment of Multi-Infarct Dementia and Alzheimer's Disease*

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ABSTRACT: Ten patients with severe dementia due to Alzheimer's disease (AD) or multi-infarct dementia (MID) or both, were treated with the precursor amino acids of the neurotransmitters serotonin and dopamine. The precursor amino acids (PAA) were given orally in a preparation that included tyrosine (4 gm daily) and 5-hydroxytryptophan (5-HTP) (800 mg daily), plus carbidopa (100 mg daily) as an aromatic amino-acid decarboxylase inhibitor. Diagnosis was established by an electroencephalogram, brain scan, computerized axial tomographic scan, and in one case by necropsy findings. Serial clinical evaluations and measurements of neuropsychologic function were performed. Levels of homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) were determined before and after administration of probenecid. Side effects of the PAA therapy were diarrhea, drowsiness, nausea, vomiting and agitation, all of which were controlled by reducing the dosage. One patient with MID and one with AD+MID showed clinical and psychologic improvement, but the others did not improve. Analysis of the cerebrospinal fluid for HVA and 5-HIAA before and after the probenecid test indicated some improvement in the metabolic turnover of these acid metabolites of serotonin and dopamine after administration of their precursor amino acids.

At present there are no generally accepted methods for the treatment of patients with organic dementia of the two most common types, i.e., multi-infarct dementia (MID), Alzheimer's disease (AD) or admixtures of both (1–4). The present communication is concerned with a trial of 5-hydroxytryptophan, tyrosine and carbidopa therapy in these dementias.

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In MID, it is possible that higher cortical function becomes deranged because of multiple zones of infarction, reduced blood flow and energy production, with depletion of the neurotransmitters (5–8). In MID patients, computer-assisted tomography (CT scans) may aid in the differential diagnosis by showing irregular parenchymatous atrophy or asymmetrical ventricular enlargement; and noninvasive measurements of regional cerebral blood flow (rCBF) by means of the ^{133}xenon inhalation technique may reveal patchy reduction of rCBF (4).

The second category involves patients with presenile and senile cerebral parenchymatous atrophy, of which the most common type is AD. In AD, probably the reduction of CBF and energy production is secondary to a primary neuronal metabolic disorder, rather than the cause of it, since the regional neurofibrillary deprivation of neurons and atrophy of the brain are most marked in the frontal and temporal zones where neurotransmitter metabolism and synthesis appear to be maximally disordered (4, 9–16). In AD, the CT scans generally show symmetrical atrophy in the fronto-temporal and parietal regions, with corresponding patterns of reduced blood flow.

The natural history of both these disorders is progressive deterioration with tragic loss of personal dignity. Such patients pose an enormous drain on the economic and domiciliary resources of society. In both these categories, available evidence indicates that the severity of the dementia is due to neuronal atrophy in selected areas of the brain (comparable to the process in Parkinson's disease) rather than to a gross loss of cerebral tissue. On the basis of this hypothesis, treatment of both AD and MID by oral replacement of the precursor amino acids that might increase the availability of deficient neurotransmitters warrants investigation. The objectives of the present study were to see whether such therapy might result in improved neurologic, psychologic and electrophysiologic functions correlated with evidence of improved neurotransmitter synthesis measured in the cerebrospinal fluid (CSF) of representative patients with these disorders.

MATERIAL AND METHODS

Design of therapeutic trial

Ten patients with severe dementia due to Alzheimer's disease (AD), multi-infarct dementia (MID) due to cerebrovascular disease, or a combination of the two (AD plus MID), were admitted to the trial of therapy with the precursor amino acids (PAA), tyrosine and 5-hydroxytryptophan (5-HTP) plus carbidopa (an aromatic amino-acid decarboxylase inhibitor). Four patients had AD alone, 3 had dementia of the multi-infarct type, and 3 had dementia of the mixed type (AD plus MID). Diagnosis was confirmed by clinical evaluation, routine hematologic and biochemical studies, an EEG, a brain scan and a CT scan. In 6 cases four-vessel angiography, in 2 cases pneumoencephalography, and in 1 case ^{169}ytterbium ventriculocisternography were also carried out after infusion into the lumbar subarachnoid space, to exclude the diagnosis of normal-pressure hydrocephalus. In Patient #2, the diagnosis of cerebral atrophy with marked cortical neuronal cell loss, degeneration and astroglial proliferation was confirmed at necropsy.

Further assessment was performed according to the approved protocol. This included clinical evaluations by the attending physicians and nursing staff, rCBF measurements in 4 patients by the ^{133}xenon inhalation technique, cortical evoked responses, neuropsychologic examination, and measurement of cerebrospinal fluid homovanillic acid (HVA), 5-hydroxyindoleacetic acid (5-HIAA) and in some cases cyclic AMP before and after administration of probenecid.

The CT scans in all patients showed gross evidence of cerebral cortical atrophy. In MID, focal parenchymatous zones of low photon density usually were present, presumably due to remote infarctions. In AD, the atrophy of the cortex and enlargement of the ventricles usually were symmetric. In AD plus MID, the brain atrophy was diffuse but there was a clinical history of focal cerebrovascular disease or atherosclerotic plaques as demonstrated by angiography.

Patients

The sample studied consisted of 5 men and 5 women whose ages ranged from 48 to 82, with a mean of 63.6 years (Table 1). The mean age for the men was 55.4 years, and for the women 71.8 years. In 3 of the 5 men, the diagnosis was MID, in one it was AD, and in one it was AD + MID. In 3 of the 5 women, the diagnosis was AD, and in 2 it was AD + MID (Table 1).

PAA therapy; experience with dosage

All 10 patients received a combined preparation consisting of tyrosine, 5-HTP and carbidopa

(MD-486). In each case an attempt was made to initiate daily therapy at the level of tyrosine 4 gm, 5-HTP 800 mg, and carbidopa 100 mg.

In practice, it became apparent that the optimal maintenance dosage was half to one-quarter of that arbitrarily proposed in the original protocol. Only Patient #7 was able to tolerate the full dosage. In the others, the dosage was reduced to the point of tolerance.

Duration of trial

In 2 of the 10 patients admitted to the study, the PAA therapy was discontinued after 6–12 weeks because there was no therapeutic benefit. In 4 patients the PAA therapy was discontinued because of side effects. However, the 4 remaining patients were treated on a long-term basis, with side effects controlled through adjustment of the dosage. Of these 4 patients, one (Patient #7) showed marked clinical improvement, one (Patient #6) moderate clinical improvement, and two (Patients #4 and #9) showed no deterioration. In Patient #9, the PAA preparation had to be discontinued after six months, but treatment is continuing in Patients #4, #6 and #7.

RESULTS – CLINICAL COURSE

Details of the course in each patient are available in special charts and data sheets as well as in medical records available from the Department of Neurology and The Methodist Hospital.

No clinical improvement – 8 patients

Patient #1 (see Table 2 for numbers). Severe MID. This 61-year-old hypertensive male had a history of strokes resulting in cortical blindness, disorientation, confusion and restlessness. The CT scan showed brain atrophy with bilateral focal zones of low x-ray density. Therapy was discontinued after three months of maintenance with a daily dosage of tyrosine 2 gm, 5-HTP 400 mg, and carbidopa 50 mg. He was extremely drowsy with the initial high dosage but no other side effects were noted after it was reduced by half. The clinical picture was one of restlessness, agitation and disorientation prior to the initiation of precursor amino-acid therapy. No clinical improvement was noted and consequently treatment was not continued after the three-month trial was completed.

Patient #2. Advanced AD. This 57-year-old man had a two-year history of progressive de-

TABLE 1
Sex, Age, and Diagnostic Distribution

Sex and Number	Age Range (yrs.)	Mean Age (yrs.)	Clinical Diagnosis
Males			
5	48–61	55.4	3 MID 1 AD 1 AD + MID
Females			
5	65–82	71.8	3 AD 2 AD + MID
Total			
10	48–82	63.6	3 MID 4 AD 3 AD + MID

mentia, shuffling of gait and memory loss, complicated by a fractured hip. The CT scan showed marked symmetrical atrophy of the brain with enlarged ventricles and widening of the sulci. The diagnosis of severe cortical atrophy with neuronal loss was confirmed at autopsy. PAA therapy was discontinued after six weeks because there was no improvement in his mental state, he had had two generalized seizures, and extrapyramidal rigidity had progressed. The seizures were controlled with anticonvulsant drugs, and the rigidity with low doses of levodopa. The two seizures probably were not due to therapy but to fat emboli from the fractured hip or the natural course of the disease. After discontinuing PAA, the patient remained severely demented and lethargic. He died six months later of bronchopneumonia. Brain atrophy with severe cortical neuronal degeneration was confirmed at autopsy.

Patient #3. Advanced AD. This 82-year-old woman had a three-year history of progressive loss of memory and confusion, but no history of strokes, risk factors for arteriosclerosis or heart disease. PAA therapy was discontinued after ten weeks when the patient was transferred from her home to a nursing home where the administration of PAA was not possible. During treatment, the side effects of nausea and vomiting were controlled by maintaining the dosage at half the proposed level. Because of drowsiness, the daily dose was reduced, one week after discharge, to 1.5 gm tyrosine, 300 mg 5-HTP and 50 mg carbidopa. The patient's family reported improvement initially in mentation and behavior, but she was troubled by urinary incontinence. At the time of transfer to a nursing home her memory had once again deteriorated and incontinence persisted.

Patient #5. Advanced AD. This 70-year-old woman had a ten-year history of progressive de-

mentia, tremor, restlessness, myoclonic jerks and tachypnea. PAA therapy was discontinued after six weeks because there was no improvement in her condition. Some diarrhea was associated with PAA administration, but marked hyperventilation and dyskinetic movements of the face and tongue with dancing movements of the lower limbs were also noted. The involuntary movements were considered to be the result of discontinuing the long-term use of haloperidol, which had caused tardive dyskinesia. However, administration of precursor amino acids may have exacerbated this condition. When haloperidol was started again and PAA therapy discontinued, the patient resumed her pre-PAA trial status.

Patient #8. Combined AD and vertebrobasilar insufficiency. This 56-year-old man had a four-year history of progressive memory loss, flattened affect and dyscalculia with nocturnal hallucinations. Brain atrophy had been shown by pneumoencephalogram three years previously. PAA therapy was discontinued after five weeks due to side effects with a daily dosage of tyrosine 2 gm, 5-HTP 400 mg, and carbidopa 50 mg. The side effects were agitation, increased confusion, occasional hallucinations and episodes of hyperventilation. His neurologic status after discontinuing PAA remained the same as before its administration.

Patient #10. Advanced AD. This 73-year-old white woman had a four-year history of progressive memory loss and inability to speak or remember names of family and friends. PAA therapy was discontinued after four and a half months. The daily dose had been tyrosine 1.5 gm, 5-HTP 300 mg, and carbidopa 50 mg. A higher dosage produced side effects (drowsiness, nausea, vomiting and diarrhea), all of which disappeared when the dosage was reduced. No clinical improvement was noted either subjectively or objectively in her neurologic or behavioral performance either at home or in the hospital setting.

Patient #4. Combined AD and cerebrovascular disease. This 65-year-old woman had motor and receptive dysphasia, echolalia, memory loss and confusion. Arteriography showed marked cerebral arteriosclerosis. Initially, side effects developed with the full dosage of PAA, including loss of weight, sleeplessness and diarrhea. With a decreased dosage—tyrosine 2 mg, 5-HTP 400 mg and carbidopa 50 mg daily—there were no side effects and no change in mentation or dysphasia, but the patient was less restless.

Patient #9. MID. This 55-year-old hypertensive male had a two-year history of memory loss, episodic hemiparesis and focal seizures. Myocardial infarction had occurred 12 years previously. Initially, the full dosage of PAA was well tolerated. Three weeks after reaching the maintenance level, side effects appeared—severe diarrhea and feelings of acute depersonalization, aggression and behavioral activation. The patient also complained of episodes of generalized weakness and paresthesias which had been present before the beginning of therapy. The dosage of PAA was reduced and he was maintained with tyrosine 1.0 gm, 5-HTP 200 mg and carbidopa 25 mg daily for six months.

Clinical improvement—2 patients

Patient #6. MID. This 46-year-old diabetic and hypertensive male had a history of multiple strokes, with repeated episodes of disorientation and slurred speech, left hemianopia, memory loss and cortical sensory impairment. Initially, he was maintained with a full dosage of tyrosine 4 gm, 5-HTP 800 mg and carbidopa 100 mg daily, which he tolerated well until six weeks after discharge. Then he noted diarrhea, drowsiness, nausea, and episodes of a floating sensation. He was less aggressive but he discontinued PAA for about six weeks because of an upper respiratory infection with fever. PAA therapy was cautiously reinstituted at a half-dosage level, which he tolerated well for about four months. Then treatment was discontinued because of dizziness and confusion (as though "drunk"), but restarted at a lower daily dose (tyrosine 1.0 gm, 5-HTP 200 mg and carbidopa 50 mg) with good tolerance. This patient showed significant improvement in mentation and overall performance in the home. He was less irritable and could take care of his daily self-care in the home with minimal supervision by the family. His memory and judgment improved, as did the left homonymous hemianopsia and thalamic pain.

Patient #7. Combined AD and vertebrobasilar insufficiency. This 69-year-old housewife had a history of progressive memory loss, resting tremor, rigidity and myoclonic jerks. Her sister had AD. Brain atrophy was demonstrated by pneumoencephalography, and advanced cerebral arteriosclerosis by arteriography. This patient was the only one of the original 10 to be maintained with a full dosage of precursor amino acids. At first, she had occasional diarrhea but soon tolerated the treatment well. Her mentation, particularly for recent memory, improved. She now can remember telephone numbers and

grocery bills—something she was unable to do previously. Involuntary movements, particularly myoclonus of the lower limbs and upper limbs ceased, as did the generalized rigidity and hemiparkinsonian features. The patient's husband and friends state that she is healthier than at any time during the past ten years. The only possible side effect was urinary frequency but this has resolved during the past 12 months.

RESULTS OF SPECIAL LABORATORY TESTS

Probenecid test

The cerebrospinal fluid (CSF) levels of HVA and 5-HIAA in terms of ng/ml of spinal fluid before and after probenecid are shown in Table 2. In all but one instance (Patient #10) the HVA levels in the CSF were low in the initial steady state, and in 5 cases there was evidence of decreased turnover of HVA. In 3 patients (Nos. 1, 5 and 7) there was competitive inhibition of tyrosine uptake by 5-HTP at the blood-brain barrier, resulting in decreased central dopamine metabolism, as judged by the probenecid test before and after PAA therapy. In these 3 patients, baseline (pretreatment) HVA levels were low, and there was a normal increase after probenecid in one instance (Patient #1).

In 2 additional patients (Nos. 8 and 10), both HVA and 5-HIAA levels in the CSF and the increase after probenecid administration were considered normal before treatment with PAA. After PAA treatment there was some increase in 5-HIAA levels and turnover with the probenecid test, but little change in HVA levels, possibly related to competitive inhibition.

In 4 patients (Nos. 2, 4, 6 and 9), both central dopamine and serotonin metabolism were impaired before PAA therapy, as judged by low steady-state levels of HVA and 5-HIAA in the CSF or absence of an increase after probenecid. There was evidence for normalization or improvement in both systems, on serial testing after PAA therapy. It is noteworthy that during therapy, 5-HIAA rose at a much more rapid rate than did HVA in all 10 patients, suggesting a competitive inhibition of tyrosine by 5-HTP. It is also of interest that in Patients 4, 6 and 9, both HVA and 5-HIAA levels rose during the first few weeks of therapy and then tended to decrease but remained above baseline levels. Probably this was due to the larger doses of the PAA administered initially in Patients 4, 6 and 9. In Patient #10, HVA levels were normal initially and did not change after PAA administration.

Likewise, Patient #3 showed decreased serotonin and dopamine metabolism with improvement in serotonin metabolism after PAA replacement therapy, but no improvement or possibly deterioration in dopamine metabolism. This also was consistent with competitive inhibition.

Cerebrospinal fluid levels of cyclic AMP were measured in 4 patients. The levels were normal in 3 of the 4; the exception was Patient #6, whose cyclic AMP increased after probenecid was reduced.

In summary, analysis of the cerebrospinal fluid for HVA and 5-HIAA levels before and after the probenecid test in all 10 patients showed some evidence of competitive inhibition of tyrosine uptake at the blood-brain barrier by administration of 5-HTP.

Neuropsychologic evaluation

In all patients the following series of tests was attempted: Wechsler Adult Intelligence Scale, Wechsler Memory Scale, Aphasia Screening Test, Tapping Test, Form Board, Drawings on Command, Sensory-Perceptual Examination, and Trail Making Test.

Patients 1, 2 and 5 were so severely demented that reliable quantitative data were unobtainable, but the test results indicated severe and generalized organic cerebral dysfunction. Repeated examinations of these patients after PAA administration were not completed because the findings would not permit quantitative comparison.

In all the remaining patients, testing was repeated after PAA therapy. In Patients 6, 7, 9 and 10, a complete series of tests was performed. In Patients 3, 4 and 8, testing was performed after PAA therapy by techniques selected on the basis of the patient's ability to cooperate and judged to provide reliable quantitative evidence of impairment when compared to the initial test performance. Two evaluations were obtained in 4 patients (Nos. 3, 4, 8 and 10), three in 2 patients (Nos. 7 and 9), and four in one patient (No. 6).

The only 2 subjects showing improvement in neuropsychologic performance after therapy were Patients 6 and 7. Patient #6 (MID) was evaluated at intervals of two, nine, and sixteen weeks after initiation of therapy. Significant overall improvements were noted with the successive tests, particularly with respect to cognition and memory, and this correlated well with the clinical

TABLE 2

Effects of Precursor Amino-Acid Therapy on HVA and 5-HIAA Levels in CSF Before and After Probenecid

Patient No.	Diagnosis	Duration of Therapy	Before Probenecid (ng/ml/CSF) HVA	Before Probenecid 5-HIAA	Before Probenecid cAMP	After Probenecid (ng/ml/CSF) HVA	After Probenecid 5-HIAA	After Probenecid cAMP	Comments
1	MID	None	17.6(↓)$^{\downarrow}$	18.8(↓)$^{\downarrow}$	19.0 (N)^{N*}	155.9	78.5^{N}	42.4^{N}	Normal findings. Steady-state values low with good response to probenecid
		2.5 mos.	Trace$^{\downarrow\downarrow}$	21.1$^{\downarrow}$		Trace$^{\downarrow\downarrow}$	55.8^{N}		Marked impairment of DA turnover. Competitive inhibition of tyrosine by 5-HTP
2	AD	None	Trace$^{\downarrow\downarrow}$	59.1^{N}		46.2^{N}	50.7 (no rise)		Impaired DA and 5-HTP turnover
		5 wks.	15.3$^{\downarrow}$	46.9^{N}		279.4^{N}	175.0^{N}		5-HTP turnover now normal. DA turnover still impaired but improved
		2 mos. (L-dopa × 2 wks)	10.8$^{\downarrow}$	27.2^{N}		Only 1 l. puncture			DA turnover still impaired despite of L-dopa therapy
3	AD	None	ND$^{\downarrow\downarrow}$	12.5$^{\downarrow}$		140.4^{N}	31.3^{N}		Normal DA turnover despite low steady-state value. Moderate impairment of 5-HTP turnover
		2 wks.	ND$^{\downarrow\downarrow}$	51.4^{N}		40.4$^{\downarrow}$	82.9^{N}		
4	AD + MID	None	19.4$^{\downarrow}$	12.2$^{\downarrow\downarrow}$		19.4$^{\downarrow\downarrow}$	12.2$^{\downarrow\downarrow}$		Severe impairment of both DA and 5-HTP turnover
		2.5 wks.	73.7^{N}	117.9$^{\uparrow}$		161.8^{N}	216.6^{N}		Normal values
		4 mos.	21.0^{N}	61.6^{N}		123.0^{N}	183.1^{N}		Normal values
5	AD	None	16.7$^{\downarrow}$	31.2		83.2^{N}	76.9^{N}		Mild impairment of DA turnover. Normal 5-HTP turnover
		3.5 wks.	Trace$^{\downarrow\downarrow}$	58.4^{N}	19.6^{N}	60.6^{N}	192.0^{N}	29.8^{N}	Competitive inhibition of tyrosine by 5-HTP
		1.5 mos.	23.6^{N}	22.7^{N}		Only 1 l. puncture			DA turnover now normal. Less stimulation of 5-HTP metabolism
6	MID	None	Interference	30.5^{N}	22.0^{N}	25.6$^{\downarrow\downarrow}$	46.1$^{\downarrow}$	26.4$^{\downarrow}$	Severe impairment of DA turnover. Minimal impairment of 5-HTP turnover
		7 days	Interference	133.4$^{\uparrow\uparrow}$	13.8$^{\downarrow}$	Interference	133.4 (no rise)	21.6^{N}	Maximalization of central 5-HTP turnover
		3.75 mos.	59.0^{N}	117.2$^{\uparrow}$		38.0	98.4		Could not interpret "after probenecid" data since percent probenecid in CSF did not rise. Steady-state values indicate normal DA metabolism
7	AD + MID	None	Trace$^{\downarrow\downarrow}$	26.8^{N}		25.8$^{\downarrow}$	40.3^{N}		Impaired DA turnover
		9 days	9.9$^{\downarrow}$	66.6^{N}		24.8$^{\downarrow}$	115.2^{N}		No significant change in DA turnover. 5-HTP turnover improved.
		15 wks.	↓	248.4$^{\uparrow\uparrow}$		10.0	222.5		Could not interpret "after probenecid" data since percent probenecid unchanged. Steady-state values indicated competitive inhibition
		30 wks.	43.8^{N}	248.0$^{\downarrow\downarrow}$		Only 1 l. puncture			Normalization of central DA metabolism
8	AD + MID	None	37.6^{N}	25.0^{N}	24.6^{N}	70.2^{N}	50.0^{N}	71.4^{N}	Normal findings
		3.5 wks.	42.5^{N}	57.5^{N}		Only 1 l. puncture			Normal steady-state values
9	MID	None	9.3$^{\downarrow}$	ND$^{\downarrow\downarrow}$		16.8$^{\downarrow}$	10.9$^{\downarrow}$		Severe impairment of 5-HTP and DA turnover
		12 days	57.0^{N}	107.6$^{\uparrow}$		117.7^{N}	122.0^{N}		Marked stimulation of both 5-HTP and DA turnover
		4 mos.	21.6^{N}	30.7^{N}		48.0^{N}	57.3^{N}		Normal findings
10	AD	None	42.9^{N}	33.6^{N}		196.0^{N}	151.6^{N}		Normal findings
		10 days	46.1^{N}	199.0		225.3^{N}	491.1^{N}		Increase in both DA and 5-HTP metabolism
		4.5 mos.	43.7^{N}	157.8		134.0^{N}	271.0^{N}		Stimulation of 5-HTP. DA normal

* N = normal.

observations. Patient #7 (AD plus MID) was tested at intervals of fifteen and thirty weeks. The overall changes in neuropsychologic performance were in the direction of minimal improvement; the clinical status showed marked improvement.

No significant changes in neuropsychologic performance were noted in Patients 3, 4 and 9, and mild decrements in overall performance were observed in Patients 8 and 10. In Patient #8, the decrement in neuropsychologic performance was associated with agitation, increased confusion and occasional hallucinations observed clinically.

Regional cerebral blood flow

Satisfactory measurements of rCBF and blood volume (rCBV) were made in 4 patients (Nos. 4, 7, 9 and 10) with use of the noninvasive xenon-133 inhalation method.

Patient #4 (AD plus MID) showed reduction of mean cerebral blood flow in both frontal regions, more marked in the left posterior frontal region where the fast component (flow in gray matter) and mean flow values were severely reduced, compatible with the clinical findings of motor dysphasia and echolalia. Regional CBV was also decreased.

Patient #7 (AD plus MID) showed bilateral and diffuse reduction of rCBF and rCBV in both hemispheres, which was maximal in both parietal and posterior temporal regions where the gray-matter flow was most severely reduced.

Patient #9 (MID) showed asymmetrical focal reductions of mean regional flow in both posterior temporal and parietal regions, with bordering hyperemia. The reduction of rCBF was primarily in white matter. Regional CBV was significantly reduced only in the left cerebral hemisphere.

Patient #10 (AD) showed reduction of mean rCBF in the posterior frontal and parietal regions bilaterally, most marked in the parasagittal areas.

In one patient (No. 7), serial tests were performed fifteen and thirty weeks after starting PAA therapy. Both tests showed good agreement, with significant focal increases of flow in the left posterior parietal, posterior temporal, right frontal, and posterior temporal regions.

Cerebral evoked responses

Methods. Separate visual, auditory and somatosensory stimuli were presented to all the patients before and after PAA therapy. The subject was seated comfortably in a dimly lit room with the eyes closed. The electrode positions were arranged according to the 10–20 system. Responses were obtained from monopolar leads (earlobe reference).

By means of an on-line computer, the responses obtained from 128 sequential stimuli were averaged and the calculated average response was considered to be the response to a given test, whether visual, auditory or somatosensory. All patients were examined before and after PAA therapy.

Results. In general, the evoked responses in all patients were abnormal in waveform compared to those of a control group of 12 normal subjects. Responses were measured as changes observed in amplitude, distribution and latency of identifiable components. The trend of change initially observed for these patients was as follows:

Visual evoked responses. Four patients (Nos. 1, 2, 8 and 9) showed an increase in amplitude of the visual evoked responses after therapy. Three patients (Nos. 4, 6 and 10) showed a decrease in amplitude. No patient showed a decrease in latency of visual evoked response.

Auditory evoked responses (AER). Two patients (Nos. 6 and 9) showed an increase in AER after therapy, whereas 2 patients (Nos. 7 and 8) showed a decrease. The others showed no change in amplitude, and no patient showed a decrease in latency of AER.

Somatosensory evoked responses (SSER). Three patients (Nos. 4, 8 and 9) showed an increase in the amplitude of SSER after therapy. No patient showed a decrease in latency.

In summary – There was no consistent pattern of change as judged by decreased latency of cerebral evoked responses. In general, the patients showing a trend toward an increase in amplitude of visual evoked response components [identified as waves III-IV (Ciganek)] showed little or no evidence of clinical improvement. However, the 2 patients (Nos. 6 and 7) who improved clinically were tested four times, revealing consistent serial changes in specific features of late components of the evoked response for both the visual and the auditory modality. For the AER, Patient #7, who alone was maintained with the full dosage of PAA, showed serially increasing right/left asymmetry in the amplitude of the late components of the auditory response.

Likewise, for the visual response, a serially increasing amplitude of a late negative component (at 200 msec latency) was noted for Patient #7. This result contrasted with changes observed

• N = normal.

in the amplitude of a major peak characterizing the early phase of the response. The early peak (at 70 msec latency) showed some decrease in amplitude following therapy.

Patient #6 initially was maintained with the full dosage of PAA, but this dosage was later reduced, and still later reduced again. His initial post-therapy auditory evoked response revealed a very large increase in amplitude as compared to the pre-therapy value, but the late components of this response became attenuated serially for the three post-therapy sessions. A similar post-therapy serial attenuation of some late components (beyond 300 msec latency) was noted in the visual evoked response for this patient, at a point where the initial post-therapy amplitude of these late components showed an increase over the pre-therapy control values.

SIDE EFFECTS OF ORAL ADMINISTRATION OF TYROSINE, 5-HTP AND CARBIDOPA

Diarrhea. The most frequent side effect of this combination of precursor amino acids (PAA) was diarrhea. Diarrhea occurred in every patient upon initial institution of PAA therapy. All patients, when maintained with a daily dosage of tyrosine 2 gm, 5-HTP 400 mg and carbidopa 50 mg, occasionally had frequent stools. When the dosage was lowered the frequency was reduced.

Drowsiness. Some drowsiness occurred in 70 percent of the patients while they were receiving PAA therapy in full dosage, but it disappeared with reduction in dosage. It appeared to be a side effect of 5-HTP.

Nausea and vomiting. Nausea was frequent during the initial institution of PAA therapy. Vomiting was rare and was usually related to the administration of probenecid for examination of the dopamine and serotonin turnover in the CSF.

Behavioral changes. Restlessness and agitation were noted with high dosages in 50 percent of the patients. This was associated with confusion and hallucinations in one patient (No. 8), and 2 patients (Nos. 6 and 9) complained of feeling different or "strange." These symptoms were promptly relieved by lowering the dosage. Hyperventilation occurred in 2 patients (Nos. 7 and 8) but ceased on reducing the dosage or discontinuing PAA. Seizures were seen in one patient (No. 2), who entered with a fractured hip. These seizures were of the grand mal type and were probably not related to PAA therapy but to cerebral fat embolization.

DISCUSSION

These attempts at oral replacement therapy for the cerebral neurotransmitters serotonin, dopamine and norepinephrine were made through use of a combination of their precursor amino acids, tyrosine and 5-HTP, plus the amino acid decarboxylase inhibitor carbidopa. The observations were carried out in 10 patients with severe dementia due to AD, or MID due to cerebrovascular disease, or both.

An attempted initial oral daily dosage of tyrosine 4 gm, 5-HTP 800 mg and carbidopa 100 mg proved to be excessive. It produced side effects of frequent stools or diarrhea in all patients, nausea in the majority, drowsiness in 70 percent, and agitation, confusion and restlessness in 50 percent. All these symptoms were controlled by lowering the dosage to half or one-quarter of that proposed in the original protocol. Clinical evidence of improvement in neurologic and clinical status, behavior, and performance in the home and society was noted in 2 patients, and this improvement has continued with PAA therapy for as long as 12 months. One other patient has continued therapy for 7.5 months without any change in her status. In the remaining 7 patients, PAA therapy was discontinued after five weeks to seven months (mean, four months) because of lack of improvement or because of side effects. No patient showed permanent ill-effects or neurologic deterioration while participating in the therapeutic trial.

Serial examination of the CSF levels of 5-HIAA and HVA showed that PAA therapy consistently increased serotonin turnover, but caused depression or less elevation of dopamine turnover, apparently due to competitive inhibition of tyrosine uptake at the blood-brain barrier by 5-HTP.

Neuropsychologic testing showed significant improvement in overall performance in one MID patient who also had improved clinically. Likewise, the overall change in neuropsychologic performance in the direction of minimal improvement in one patient with AD + MID was confirmed by the clinical findings which indicated striking improvement in neurologic status. The other patients did not improve.

Studies of visual, auditory and somatosensory evoked responses showed no consistent decrease in latency or increase in amplitude of the responses, based on generalized waveform comparisons of the pre- and post-therapy records. To this extent, cerebral evoked responses in this group of patients with moderate to severe dementia was

poorly correlated with the behavioral observations. However, interesting correlations between behavioral observations and PAA therapy were obtained for the amplitude of specific late components of the auditory and visual evoked response. Late components of the response are the ones considered to be related to information processing and higher cortical function (i.e., the contingent negative variation and P300 waves discussed extensively in the literature). Furthermore, a serially increasing right/left response asymmetry was noted for late components in Patient #6, who showed clinical improvement under conditions of continued full dosage of precursor amino acids. Callaway (17) has reported a positive correlation between right/left asymmetry of evoked response amplitude and the intelligence quotient. These observations suggest that changes in amplitude or distribution between hemispheres of late evoked response components may be related to the disease process in dementia or to the efficacy of the therapy program introduced in this study.

Regional CBF measurements showed a reduction compatible with findings previously reported in 4 cases of AD and MID. Serial measurements were obtained in only one patient (No. 7); these showed regional increases in CBF during PAA therapy as the patient improved clinically.

In this preliminary study, it appears that the disease process was too advanced in several patients (Nos. 1, 2 and 4) for proper evaluation of any effects of PAA replacement therapy. In future studies, case selection should be limited to patients with only mild or moderately advanced disease who can cooperate fully with the procedures (e.g. pyschologic testing) and administration of PAA. Nevertheless, it is encouraging that 2 patients, one in each category of the major causes of dementia (AD and MID), showed improvement in otherwise incurable and progressive disorders of the central nervous system.

A consistent observation was that the combination of tyrosine and 5-HTP with carbidopa caused competitive inhibition of tyrosine uptake by 5-HTP at the blood-brain barrier. Possibly a better balance of precursor amino acid therapy might have been achieved by the administration of levodopa and 5-HTP, or alternatively tryptophan and levodopa. The original dosage proved to be excessive, and in any future studies a lower initial dosage is recommended.

All patients with AD, MID (or both) showed low values for dopamine metabolism. For MID, this observation is in keeping with experimental observations in animal models and in patients with acute cerebral infarction, to the effect that norepinephrine and dopamine become depleted or displaced from ischemic brain tissue (5–7). In AD, no experimental model is available, but low CSF levels of HVA and 5-HIAA have been reported in AD by Gottfries et al (10, 12). Furthermore, turnover studies performed by means of the probenecid test have shown reduced dopamine metabolism and less significant reduction of serotonin metabolism in the same patients. Likewise, Parkes et al (11) have reported low levels of HVA and 5-HIAA in the CSF of patients with the akinetic and rigid type of parkinsonism associated with dementia.

In future, it may be possible to select precursor amino-acid therapy according to individual patterns of biochemical abnormality determined by measurements of HVA and 5-HIAA before and after the probenecid test. For example, if HVA metabolism is reduced, levodopa would be indicated; if 5-HIAA metabolism is reduced, 5-HTP would be indicated; and if the metabolisms of both are reduced, combination therapy would be indicated.

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ACTH, α-MSH and β-LPH: pituitary hormones with similar activity in an amnesia test in rats

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In addition to its classical endocrine effect, adrenocorticotrophic hormone (ACTH) also influences behaviour through direct effects on the brain. Thus its administration to rats during the extinction of shock-motivated responses (Greven & de Wied, 1973) or food-motivated responses (Garrud, Gray & de Wied, 1974) delayed the extinction. Extirpation of the adrenal gland did not abolish the behavioural effect of ACTH (Miller & Ogawa, 1962). Moreover, fragments, e.g. ACTH$_{4-10}$, and analogues lacking the endocrine activity of ACTH still appeared to have behavioural actions similar to the parent hormone (Greven & de Wied, 1973; de Wied, Witter & Greven, 1975). Besides, corticosteroids in general have been found to exert behavioural effects opposite to those of ACTH-like peptides (Bohus, 1970; van Wimersma Greidanus, 1970; Garrud & others, 1974).

Using the pole jump test, de Wied and associates sought the smallest amino acid sequence of ACTH that possessed essentially the same behavioural activity as the parent hormone and found ACTH$_{4-7}$ to meet the essential requirements for the inhibitory effect on extinction (Greven & de Wied, 1973; de Wied & others, 1975). This amino acid sequence is shared by several pituitary hormones, including melanocyte-stimulating hormone (MSH). Both α-MSH and porcine β-MSH affect extinction of shock-motivated (Greven & de Wied, 1973) and food-motivated responses (Kastin, Sandman & others, 1975) in the same way as ACTH.

In addition to extinction of shock- or food-motivated responses, several other paradigms have been used to assess the behavioural effects of ACTH-like peptides. We have studied the effect of ACTH$_{4-10}$ on carbon dioxide (CO_2)-induced amnesia for a one-trial passive avoidance response. When during the so-called acquisition trial of the passive avoidance test a rat is punished by an electric footshock for performing a preferred response, i.e. entering a dark chamber, it usually avoids making that response again on a subsequent trial (retrieval trial). CO_2 was able to induce amnesia for this passive avoidance behaviour, when it was administered immediately upon the acquisition trial. The CO_2-induced amnesia could be attenuated by subcutaneous treatment with ACTH$_{4-10}$ 1 h before the retrieval trial. Given 1 h before the acquisition trial the peptide was ineffective (Rigter, van Riezen & de Wied, 1974). Since α-MSH contains the amino acid sequence ACTH$_{4-10}$, it may be assumed that it also possesses anti-amnesic activity. The present study was undertaken to test this hypothesis. In addition, we examined the effect of β-lipotrophic hormone (β-LPH), a pituitary hormone that also contains the sequence ACTH$_{4-10}$ (= β-LPH$_{47-53}$)

In each experiment 7 groups of 10 male Wistar rats, 170–210 g, were trained in the one-trial passive avoidance apparatus described by Ader, Weijnen & Moleman (1972). This consisted of a 40 × 40 × 40 cm Lucite chamber with black walls and a grid floor. A 6 cm wide, 25 cm long elevated runway protruded from the front wall of the chamber. The runway was illuminated by a 40 W lamp while the chamber was dark. When placed on the runway, a rat could enter the dark chamber through a 6 × 6 cm opening. A scrambled footshock (0·5 mA for 3 s) could be delivered through the grid floor of the chamber.

On both day 1 and day 2 of each experiment the animals received two pretraining trials during which the rat was placed at the end of the runway and the time it took to enter the chamber was recorded and defined as the step-through latency. On day 3 a single acquisition trial was given. This was similar to a pretraining trial except that the rats received a footshock (FS) 10 s after entering the chamber. Immediately after the application of the FS the animals were subjected to amnesic treatment with CO_2 (FS-CO_2 groups) or to 'sham' amnesic treatment (FS-NoCO_2 groups) according to the procedure described by Rigter & others (1974). Amnesic treatment consisted of placing the rats in a box with 100% CO_2 until respiratory arrest occurred. They were then revived by artificial respiration. Sham-treated rats were placed in an identical but air-filled box. Since our previous work has shown that CO_2 itself does not affect later performance (Rigter & others, 1974), control groups receiving CO_2 but no FS were not included in the present experiments. On day 4 (24 h after acquisition) a single retrieval trial was given and the step-through latency recorded. When a rat did not enter the chamber within 180 s, it was taken from the runway and a score of 180 s was assigned.

ACTH$_{4-10}$, α-MSH, ovine β-LPH (10 μg per rat) or saline were injected subcutaneously 1 h before the acquisition trial and/or the retrieval trial according to the schedule given in Table 1. ACTH$_{4-10}$ in a hydrochloric acid solution of pH 3·5 was diluted with saline to a concentration of 10 μg ml^{-1} and the solution neutralized to pH 7 with sodium bicarbonate before injection. α-MSH and β-LPH were dissolved in saline. β-LPH was a generous gift of Dr C. H. Li, Hormone Research Laboratory, University of California, San Francisco.

* Correspondence.

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Table 1. *The influence of ACTH$_{4-10}$, α-MSH and β-LPH (10 μg per rat) on CO_2-induced amnesia for a one-trial passive avoidance response.* (A = no avoidance. B incomplete avoidance. C compete avoidance.)

Group (n = 10)	Treatment 1 h before: acquisition	retrieval	% of rats showing: A	B	C
FS-CO_2	saline	saline	80	20	0
FS-CO_2	ACTH$_{4-10}$	saline	90	10	0
FS-CO_2**	saline	ACTH$_{4-10}$	30	60	10
FS-CO_2**	ACTH$_{4-10}$	ACTH$_{4-10}$	30	50	20
FS-NoCO_2***	saline	saline	0	30	70
FS-NoCO_2***	ACTH$_{4-10}$	saline	10	10	80
FS-NoCO_2***	saline	ACTH$_{4-10}$	0	30	70
FS-CO_2	saline	saline	100	0	0
FS-CO_2	α-MSH	saline	90	0	10
FS-CO_2*	saline	α-MSH	60	40	0
FS-CO_2†**	α-MSH	α-MSH	33·3	55·5	11·1
FS-NoCO_2***	saline	saline	30	20	50
FS-NoCO_2***	α-MSH	saline	20	0	80
FS-NoCO_2***	saline	α-MSH	0	20	80
FS-CO_2	saline	saline	70	30	0
FS-CO_2	β-LPH	saline	60	40	0
FS-CO_2†*	saline	β-LPH	33·3	44·4	22·2
FS-CO_2**	β-LPH	β-LPH	10	50	40
FS-NoCO_2***	saline	saline	0	20	80
FS-NoCO_2***	β-LPH	saline	0	20	80
FS-NoCO_2***	saline	β-LPH	0	40	60

ACTH$_{4-10}$, α-MSH and β-LPH (10 μg per rat) were studied in separate experiments.
† 1 animal lost due to CO_2. *: difference to corresponding saline-saline FS-CO_2 group: $P < 0·05$; **: $P < 0·01$; ***: $P < 0·001$.

The test scores were divided into three classes: (1) latencies of 0–10 s; (2) latencies of 11–179 s; (3) latencies of 180 s. Rats entering the chamber within 10 s were considered as not showing passive avoidance; previous experiments had shown that 10 s represented the maximum latency for rats which had not received a footshock at the time of the acquisition trial.

Rats entering the chamber between 11–179 s displayed incomplete passive avoidance while those that failed to enter within 180 s were considered to show a complete passive avoidance response. In the analysis of the results, the three classes received a weighting of 0, 1 and 2, respectively. The retrieval scores were analysed with the one-tailed Yates test (Yates, 1948).

The results are shown in Table 1. In accordance with our previous finding (Rigter & others, 1974), ACTH$_{4-10}$ attenuated CO_2-induced amnesia when administered before the retrieval trial but not when given before the acquisition trial. The group of rats treated with the peptide before both acquisition and retrieval did not differ statistically from the group receiving the peptide only before the retrieval trial. ACTH$_{4-10}$ did not change passive avoidance behaviour in the FS-NoCO_2 groups.

α-MSH and β-LPH exerted an anti-amnesic effect which was essentially similar to that of ACTH$_{4-10}$; both hormones reduced amnesia but only when given before the retrieval trial. Although treatment before both acquisition and retrieval tended to increase the anti-amnesic effect, this was not statistically significant. α-MSH and β-LPH did not affect the retrieval scores of FS-NoCO_2 rats.

These results indicate that pituitary hormones sharing the amino acid sequence ACTH$_{4-7}$ (and ACTH$_{4-10}$) have similar behavioural actions in our amnesia test. This is in agreement with the results of Greven & de Wied (1973) who demonstrated a similar inhibitory effect of ACTH, α-MSH and β-MSH on extinction of a shock-motivated response. In the present experiments ACTH$_{4-10}$, α-MSH and β-LPH were administered in a dose of 10 μg per rat. Dose-response studies are needed to assess possible differences in potency.

β-LPH has received considerable attention lately as oligopeptides derived from the C-terminal part of this hormone (amino acid sequence 61–91) appeared to have strong morphinomimetic actions (Hughes, Smith & others, 1975; Cox, Goldstein & Li, 1976; Lazarus, Ling & Guillemin, 1976;. It has been suggested that β-LPH functions as a prohormone for peptides with morphinomimetic activity (Cox & others, 1976; Lazarus & others, 1976). The present data raise the interesting question of whether β-LPH also functions as a prohormone for ACTH-like peptides.

September 6, 1976

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CONTENTS

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NOTES

813 million people freed from endemic malaria

813 million human beings have now been liberated from endemic malaria; representing 52% of the population living in the originally malarious areas of the world—such is the present score in the world malaria eradication campaign as announced by the Executive Board of the World Health Organization (WHO) recently.

On the future development of the world campaign against malaria, the WHO Executive Board is recommending the following measures to the 18th World Health Assembly which meets in May this year.

Network of services. Governments starting an all-out campaign against malaria should give priority to countrywide development of a network of rural health services to sustain the eradication programme.

Teaching about malaria. Countries in malarious areas should ensure adequate teaching about malaria in all schools of medicine and public health on both the clinical and public health aspects of the disease.

Bilateral aid: priority for malaria. International agencies and governments providing bilateral assistance should give priority support to meet the extensive material needs of malaria eradication programmes.

Increased vigilance. Governments of countries where the malaria eradication programme has reached an advanced stage should stimulate the collaboration of all medical and health personnel in constant vigilance against the re-establishment of the disease.

A gigantic programme

The world-wide malaria eradication campaign, a public health venture of unprecedented dimensions, was launched during the 8th World Health Assembly in Mexico in 1955. Its aim was nothing less than to liberate over 1,500 million human beings for ever from the risk of malaria. To carry out this gigantic undertaking, thousands of malaria teams are at work; tens of thousands of vehicles and millions of tons of supplies are being used; health workers examine hundreds of millions of blood samples, and spray hundreds of millions of houses with insecticide.

The advisory staff engaged by WHO in 1964 for the world malaria eradication campaign amounted to 400 doctors, sanitarians, entomologists and other specialists.

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Neonatal Thyroxine Stimulation Accelerates the Maturation of Both Locomotor and Memory Processes in Mice

James M. Murphy and Z. Michael Nagy
Bowling Green State University

In two experiments mice were injected with thyroxine on Postnatal Days 1, 2, and 3, and the subsequent effects upon the development of the swimming reflex and the emergence of instrumental learning/memory processes were examined. In agreement with past studies, early thyroxine treatment accelerated the maturation of swimming capacities and general physical development compared with littermate controls receiving saline injections. In the second study, thyroxine- and saline-treated mice received 25 training trials on a shock-escape T-maze task at 7, 9, 11, or 13 days of age with a retention test 24 hr later. The results indicated that while learning was equivalent within each of the ages between the treatment groups, onset of 24-hr retention capacity occurred approximately 2 days earlier in the thyroxine-treated mice than in controls. In addition, a performance deficit was observed in the thyroxine mice at the oldest age tested, in agreement with previous reports. The results of these experiments suggest that early hyperthyroidism results in earlier maturation of both locomotor and memory processes, followed by later performance deficits.

Recent experiments have indicated that the onsets of learning and memory capacities for simple instrumental tasks occur at rather specific developmental stages during the first 2 wk of life in the rat and mouse, presumably reflecting the functional maturation of physiological and/or biochemical processes underlying these capacities; for example, although within-session improvement on a shock-escape straight-alley task has been found to occur as early as 5 days of age in both mice and rats, a 24-hr retention capacity does not emerge in either species until 9–10 days of age (Misanin, Nagy, Keiser, & Bowen, 1971; Nagy, Misanin, Newman, Olsen, & Hinderliter, 1972). On the more complex T-maze task, mice demonstrate improvement in escaping to the choice point prior to 9 days of age, but within-session improvement in correct choice-point turns is not evident until 9 days of age (Nagy & Murphy, 1974). A 24-hr retention capacity for prior choice-point training does not emerge until 11–12 days of age in mice (Nagy & Murphy, 1974; Nagy, Pagano, & Gable, 1976; Nagy & Sandmann, 1973).

The rather abrupt onsets of these instrumental learning and memory capacities in neonatal rats and mice coincide with the period of most rapid central nervous system (CNS) development in these species (cf. Bass, Netsky, & Young, 1969a, 1969b; Deza & Eidelberg, 1967; Grossfeld & Shooter, 1971; Himwich, 1970) and suggest that this may be an extremely critical period for the initial development of processes underlying adultlike learning and memory capacities. If so, then a manipulation that alters the rate of CNS maturation should also show parallel effects upon the emergence of learning and memory capacities.

One procedure found to accelerate development of numerous CNS processes in the rat is the administration of thyroid hormones within the first week following birth. Accelerated maturation has been evidenced

This article is based upon data from a thesis submitted by the first author in partial fulfillment for the master's degree at Bowling Green State University. Portions of these data were presented at the 1975 meeting of the Midwestern Psychological Association. This research was supported by grants from the National Science Foundation (GB-30456) and the National Institute of Child Health and Human Development (HD-09145-01) to the second author.

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by the earlier appearance during ontogeny of dendritic spines (Schapiro, Vukovich, & Globus, 1973), indexes of myelin formation (Schapiro, 1966; Walravens & Chase, 1969), adultlike cortical electroencephalographic patterns (Schapiro & Norman, 1967), CNS protein synthesis (Dunn, 1972), acetylcholinesterase levels (Schapiro, 1968), and a general acceleration of biochemical development (Grave, Satterthwaite, Kennedy, & Sokoloff, 1973). Behavioral, motoric, and sensory capacities are also accelerated or advanced in the thyroxine-treated rat (Eayrs, 1964; Schapiro, 1971); for example, the development of adultlike swimming behavior, a general index of motor, sensory, and reflexive capacities, has been consistently reported to occur earlier in thyroxine-treated rats (Davenport & Gonzalez, 1973; Schapiro, Salas, & Vukovich, 1970).

Whereas the evidence that early hyperthyroidism results in accelerated maturation of a number of physiological, biochemical, sensory, and reflexive behavior processes is quite clear, the question whether thyroid-induced maturation also includes those processes necessary for learning and memory has produced contradictory findings. Hamburgh, Lynn, and Weiss (1964) found no effect of postnatal thyroid hormone injections in rats upon the learning of an escape response at 20–28 days of age, whereas Eayrs (1964) reported a resulting performance deficit on a closed-field test at 100 days of age. Similar results were obtained by Davenport and Gonzalez (1973), who found little difference between thyroxine-injected and control rats on active and passive avoidance learning at 19–20 days of age but superior performance by controls on a passive avoidance task at 35–36 days of age and on a closed-field maze at 70–85 days of age. In contrast to these findings which indicate that thyroxine treatment does not result in accelerated or better learning than in controls, thyroxine-treated rats have been reported to perform better than controls on an active avoidance task when tested at 16–18 days of age (Schapiro, 1968).

While these results appear conflicting, it is significant to note the relation between the effect of thyroxine treatment and the age at testing which emerges from these data. When thyroxine-treated subjects were tested prior to weaning age (16–18 days of age), they performed significantly better than did saline controls. However, when they were tested between 19 and 30 days of age, thyroxine appeared to have had little effect, whereas testing at later ages suggested that the effect of thyroxine treatment was detrimental, resulting in learning and performance deficits. This pattern fits well with the proposal that one consequence of thyroxine-induced acceleration may be a premature termination of CNS development and a subsequent decrease in adult behavioral plasticity (Schapiro, 1968, 1971).

The purpose of the present investigation was to reexamine the question whether thyroxine-induced acceleration of CNS maturation affects mainly unlearned locomotor abilities (Davenport & Gonzalez, 1973) or also includes those processes involving learning and memory (Schapiro, 1968). As the present study used mice as subjects, the first experiment examined the effects of early thyroxine injections upon the development of swimming behaviors in order to ascertain that the treatment effects were similar in mice to those reported for the rat. In the second experiment the development of learning and 24-hr memory capacity of a T-maze task was investigated to determine whether thyroxine treatment would result in the earlier emergence of either or both of these abilities. The T-maze escape task was selected because mice are capable of learning and displaying 24-hr retention on this task by 11–12 days of age (Nagy & Murphy, 1974), an age much younger than those at which thyroxine injections have been reported to have either no effects or deleterious effects upon learning abilities in the rat.

Experiment 1

Method

Subjects. The subjects were 80 Swiss-Webster mice (*Mus musculus*) born and reared in the psychology department mouse colony. Litters were housed with the mothers in 30.4 × 18 × 12.8 cm opaque polyethylene cages, with wood-chip shavings and nesting material on the floor. Except

during the testing session, the mothers remained with the pups at all times with ad lib food and water available. The colony and test rooms were maintained at 24 ± 1 °C; all testing was conducted during the light period of the 12:12 hr light/dark cycle.

Apparatus. A 60.3 × 30.5 × 29.5 cm glass aquarium, filled to approximately a 12-cm depth with clean tap water maintained at 27 ± 1 °C, served as the swimming apparatus.

Procedure. On the day following birth, litters comprised of 10 or more pups, with at least four of each sex, were chosen for treatment. After all pups in each selected litter were weighed, four males and four females were chosen with equal or near-equal body weights, while the remaining pups were discarded to maintain litter size at eight, thereby minimizing possible nutritional factors between litters. At 1, 2, and 3 days of age, two males and two females from each litter were injected ip (1 μg/g body weight) with thyroxine (L-thyroxine, Sigma Chemical Company), which was freshly prepared in saline suspension each day immediately prior to injections. The remaining four pups in each litter served as vehicle-injected controls.

Daily swimming tests were conducted between 1000 and 1200 hours from 5 to 20 days of age for all mice. Two independent raters scored head and front paw behaviors from 0 to 3 (modified from Schapiro et al., 1970), with a 0 being assigned to the most immature performance and a 3 for adultlike behavior. Head ratings were as follows: 0, nose and forepart of head below water surface; 1, nose touching but below water surface; 2, nose just clearing water surface; and 3, nose maintained well above water surface. Front paw ratings were as follows: 0, uncoordinated paddling with paws pushing away from the body, often resulting in circling; 1, paddling in a coordinated fashion with paws being pushed straight forward and down; 2, some evidence of front paw inhibition but spontaneous paddling of at least one paw; and 3, front paws in parallel extension and paddling completely inhibited with only back paws propelling the animal. Each daily swimming test lasted until each rater had indicated that a rating was obtained, usually within 10 to 20 sec. In the few instances in which raters were in disagreement, the average of the two ratings for that measure was used. Following the swimming session, the mice were placed in a warm (35 °C) plastic cage and allowed to dry before being returned to the home cage. Body weights were recorded each day through 20 days of age, and the day on which both eyes were open was noted for each subject.

Results

Body weight data were analyzed by a repeated-measures analysis of variance with group, sex, and age as the factors. Sex was not significant as a main effect, nor did it interact with other factors. A significant group effect, $F(1, 76) = 9.81$, $p < .005$, and Group × Age interaction, $F(19, 1444) = 3.71$, $p < .0005$, indicated that the thyroxine-treated subjects were gaining slightly less weight over days than were the saline controls; individual comparisons conducted between the weights at each age within the interaction showed that the groups began to differ significantly by 6 days of age, $F(1, 1444) = 3.90$, $p < .05$. The weight differential from Day 8 to Day 20 remained approximately .5 g, indicating that the group difference was primarily due to the thyroxine-injected subjects' gaining less weight during the first week of life. Eye opening for the thyroxine-injected animals occurred significantly earlier than for the saline-injected controls, $F(1, 76) = 178.36$, $p < .0005$, the respective means being 11.70 and 13.48 days of age, and males opened their eyes earlier than females, an average of 12.45 and 12.72 days of age, respectively, $F(1, 76) = 4.28$, $p < .05$.

Head and front paw ratings were separately analyzed by repeated-measures analyses of variance, the factors being group, sex, and age. The ability to maintain the head above water in an adultlike manner improved with age, $F(15, 1140) = 652.78$, $p < .0005$, reaching an asymptotic level by 13–14 days of age. Although the saline controls received somewhat higher ratings from 5 to 6 days of age, the thyroxine-treated mice developed adultlike head holding more rapidly and received higher ratings than did saline groups from 8 to 13 days of age, resulting in significant group, $F(1, 76) = 4.28$, $p < .05$, and Group × Age, $F(15, 1140) = 7.65$, $p < .0005$, effects. The analysis of the front paw ratings yielded results similar to head ratings, with significant main effects for age, $F(15, 1140) = 581.11$, $p < .0005$, and group, $F(1, 76) = 15.36$, $p < .0005$, and a Group × Age interaction, $F(15, 1140) = 8.35$, $p \leq .0005$.

The present results clearly demonstrate that the effects of early thyroxine treatment in mice are similar to those previously reported for the rat, resulting in earlier

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Experiment 2

Method

Subjects. The subjects were 320 Swiss-Webster mice maintained according to the procedures described in Experiment 1.

Apparatus. The apparatus consisted of two Plexiglas mazes, a straight-alley and a T-maze. The straight alley was 19.2 cm long; the T-maze measured 19.2 cm from end to end in both directions, with the stem portion 15.8 cm and each goal arm section 7.9 cm long. Both mazes were 3.4 cm wide and 7.4 cm high throughout, and in each, start boxes were formed by placing a removable door 5 cm from the closed end of the stem in the T-maze or from one end in the straight alley. The straight alley was placed upon a grid floor so that the grids ran parallel to the length of the alley, and the T-maze was placed so that the grids were parallel to the length of the stem and perpendicular to the length of the arms. The grid was constructed of 1-mm-diam. stainless steel rods spaced 3 mm from center to center. A constant-current ac shock source (Harvard Instrument Co., Model 3121) provided a .2-mA, 60-Hz scrambled shock to the grid floor.

Procedure. Subjects were injected with thyroxine or saline over the first 3 days of age as described in Experiment 1. Separate thyroxine and saline groups of 10 males and 10 females received 25 training trials according to a modified split-litter design at 7, 9, 11, or 13 days of age on either the straight-alley or the T-maze task. For the straight-alley-trained groups, thyroxine control (TC) group and saline control (SC) group, the object was to allow the subjects to learn to inhibit competing responses and traverse to the goal end of the alley. Thus, the SC and TC groups were provided with the opportunity to learn a component of the escape response that was analogous to the escape response in the stem portion of the T-maze, but they were not provided the opportunity to learn to make the appropriate turn at the choice point as were the T-maze, trained groups, thyroxine experimental (TE) group and saline experimental (SE) group.

Training for the SC and TC groups began with the mouse being placed in the start box facing the goal end of the straight alley. After 5 sec the door was removed, and shock was administered until the mouse reached within 5 mm of the goal end of the alley. During the 45-sec intertrial interval, the subject was held in the experimenter's closed hand. If the subject did not reach the goal within 300 sec (maximum latency), it was gently prodded to the goal, and the shock terminated. Immediately following the 25 training trials, the subject was returned to the home cage.

Training for the SE and TE groups was essentially the same except that the mouse was placed in the start box of the T-maze facing the choice point, and shock was terminated when the mouse reached within 5 mm of the end wall of either goal box on the first trial. If the subject failed to make a choice-point turn on the first trial within the maximum latency, it was gently prodded to the choice point, allowed to make a choice, and prodded to the goal if necessary. On the subsequent 24 trials, the subject was trained to the goal opposite the first-trial choice, which was defined as the "correct" choice-point turn and goal for that subject. A failure to reach the correct goal within the maximum latency period resulted in prodding to that goal.

At 8, 10, 12, or 14 days of age, all subjects received 25 additional training trials on the T-maze beginning 24 hr after completion of original training. The retraining procedure for the SC and TC groups was identical to that for the SE and TE groups on the first day of training, whereas the SE and TE groups were retrained to the goal that was correct on the original training day. Since the first trial on the T-maze was used to define the correct goal for each subject in the SE and TE groups on the first day of training, and in the SC and TC groups on the second day of training, the first trial was discarded for all sessions as a warm-up trial and was not included in analysis of the data.

Two performance measures were recorded on each trial for all subjects. The first measure was the time required to traverse the alley (escape latency) from shock onset in the start box to shock offset upon reaching the goal. Escape latencies were recorded on a running time meter to the nearest .1 sec, and any maximum latency trials were recorded as 300 sec. The second measure was the number of competing responses. In the straight alley, competing responses were defined as the number of 180° turns away from the goal end of the alley, while in the T-maze, competing responses were defined as the number of 180° turns made away from the choice point prior to the first choice-point turn on each trial. Thus, if the stem portion of the maze was reentered following a choice-point turn, no additional competing responses were recorded. Consideration was given only to competing responses before the choice point so as to provide a measure that would be analogous to that obtained with the straight-alley escape task. In addition, the goal arm entered upon first reaching the choice point was recorded for the T-maze sessions.

Results

Physical measures. Body weight and eye-opening data were in agreement with the results of Experiment 1. Analysis of the body weights indicated a significant Hormone × Age interaction, $F(14, 3808) = 1.75$, $p < .05$, again demonstrating that

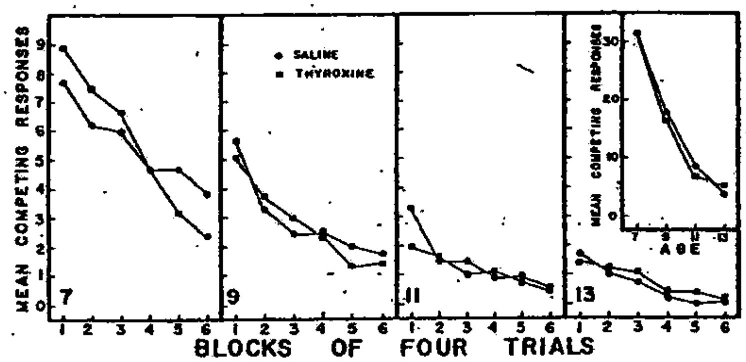

FIGURE 1. Mean competing responses on the straight-alley task as a function of hormone treatment, age, and blocks of four trials. (The figure inset shows the mean number of competing responses made for the entire training session as a function of hormone treatment and age.)

thyroxine-treated subjects gained less weight than did saline controls. The mean age that both eyes opened occurred earlier for the thyroxine-injected mice, $t(273) = 16.39$, $p < .0005$, with the mean days of eye opening being 12.34 and 13.85 for the thyroxine and saline groups, respectively.

Training. Straight alley. Two performance measures were employed for the SC and TC groups trained on the straight alley: competing responses and a speed score unconfounded by competing responses determined by obtaining the mean of the reciprocal latencies on trials without such turns. Since only a few maximum latency trials occurred, these trials were counted as any other in evaluating the performance measures.

Figure 1 presents the competing response data as a function of hormone (SC-TC), age, and blocks of four trials, while the figure inset shows the mean scores for the SC and TC groups at the four ages. A $2 \times 4 \times 2 \times 6$ analysis of variance with one repeated measure (the factors being hormone, age, sex, and trial blocks) was conducted upon these data. As can be seen in Figure 1, all groups decreased competing responses over trial blocks, $F(5, 720) = 44.16$, $p < .0005$, indicating improvement in the escape response with continued training. The younger groups made more competing responses and showed a greater decrease over trial blocks, resulting in an Age × Trial Blocks interaction, $F(15, 720) = 3.42$, $p < .0005$; evident in the figure inset is the fact that these turns decreased as a function of age, $F(3, 144) = 49.50$, $p < .0005$.

Hormone, sex, and age were the factors considered in a $2 \times 2 \times 4$ analysis of variance of the speed scores on trials without competing responses. The speed of traversing the alley increased with age, $F(3, 144) = 94.84$, $p < .0005$, and thyroxine-injected subjects ran consistently faster than saline controls of the same age, $F(1, 144) = 18.38$, $p < .0005$. Thus, although the thyroxine-treated subjects appeared to be accelerated in the maturation of the motor capacities necessary to perform the escape response, they did not differ from saline controls in the ability to eliminate competing responses in the straight alley.

T-maze. Both dependent measures for the T-maze-trained groups—competing responses before the choice point and correct choice-point turns—were analyzed by $2 \times 4 \times 2 \times 6$ analyses of variance with one repeated factor, the factors being hormone, age, sex, and trial blocks. Figure 2 presents the competing response and correct choice-point data as a function of hormone, age, and blocks of four trials, while the figure insets show the means for the SE and TE groups as a function of age.

As can be seen in the upper panel of Figure 2, all groups decreased competing responses over trial blocks, $F(5, 720) = 38.83$, $p < .0005$, and an Age × Trial

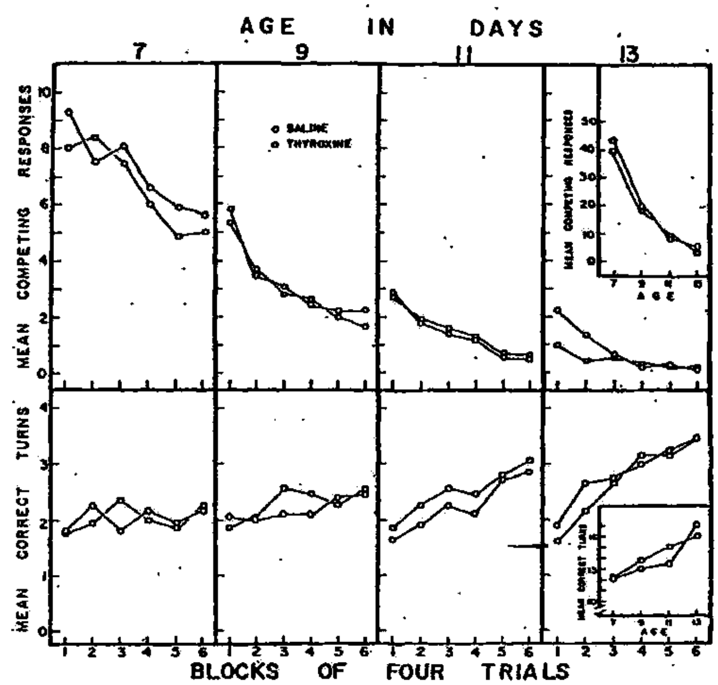

FIGURE 2. The upper panels present the mean number of competing responses made before reaching the choice point, and the lower panels present the mean number of correct choice-point turns made during original T-maze training as a function of hormone treatment, age, and blocks of four trials. (The training session means are presented in the figure insets as a function of hormone treatment and age.)

Blocks interaction is evident in the different rates of decrease for the four age groups, $F(15, 720) = 2.45$, $p < .005$. The figure inset shows the reliable decrease in competing responses with increasing age, $F(3, 144) = 80.13$, $p < .0005$. The fact that no competing response differences occurred between the SE and TE groups, $F(1, 144) < 1.00$, indicates comparable performance of that escape component for the saline- and thyroxine-treated groups.

The figure inset of the lower panel of Figure 2 illustrates the increasing ability with maturation to acquire the correct choice-point turn and, since the correct turn was defined as being opposite the first-trial choice, to overcome any initial turn preference, $F(3, 144) = 11.72$, $p < .0005$. Within-session improvement is reflected in the trial blocks effect, $F(5, 720) = 18.80$, $p < .0005$, and the greater improvement of the older groups resulted in an Age × Trial Blocks interaction, $F(15, 720) = 2.81$, $p < .0005$. Individual comparisons conducted between the first and last trial blocks for each group indicated that neither of the 7-day-old groups showed significant improvement in making the correct choice-point turn, $Fs(1, 720) \leq 2.15$, whereas all of the 11- and 13-day-old groups showed significant improvement, $Fs(1, 720) \geq 15.29$, $ps < .0005$. At 9 days of age, although both groups improved over trial blocks, the TE group, $F(1, 720) = 5.20$, $p < .025$, but not the SE group, $F(1, 720) = 1.70$, showed significant improvement in acquiring the correct choice-point turn. However, this difference proved to be as much a result of the TE group's making slightly fewer correct turns than the SE group in the first trial block (46.2% vs. 51.2%) as it was a result of the TE group's making more correct turns in the last trial block (63.8% vs. 61.2%). Moreover, comparisons conducted

between the group means within the first and last trial blocks indicated that the SE and TE groups did not differ significantly at the beginning or end of training at any of the four ages tested, $Fs(1, 720) < 1.00$, nor were there significant differences at any age when comparisons were made between group-session means, $Fs(1, 144) \leq 1.90$.

Thus, the results of the training sessions indicated little difference in performance between the thyroxine- and saline-treated groups on either task at any of the ages tested.

Retention. All groups received 25 escape training trials in the T-maze task 24 hr after the original training sessions. Dependent measures were the same as those for the original T-maze training, and a $2 \times 2 \times 4 \times 2 \times 6$ analysis of variance (the last factor a repeated measure) was conducted upon both measures. The factors were hormone, group (straight alley or T-maze trained), age, sex, and trial blocks. Upper panels of Figure 3 present the number of competing responses before the choice point, while the lower panels represent correct choice-point turns as a function of hormone treatment, group, age, and trial blocks. The figure insets show the session means as a function of hormone, group, and age.

Three significant effects common to the results in the analyses of both dependent measures will be presented collectively. An examination of the Figure 3 insets makes it clear that the age effects were a result of improved performance due to maturation, $Fs(3, 288) \geq 109.01$, $ps < .0005$. All groups improved in performance of the escape response over trial blocks, $Fs(5, 1440) \geq 50.92$, $ps < .0005$, and the differential rates of improvement for the four age groups resulted in Age $\times$ Trial Blocks interactions, $Fs(15, 1440) \geq 4.12$, $ps <$

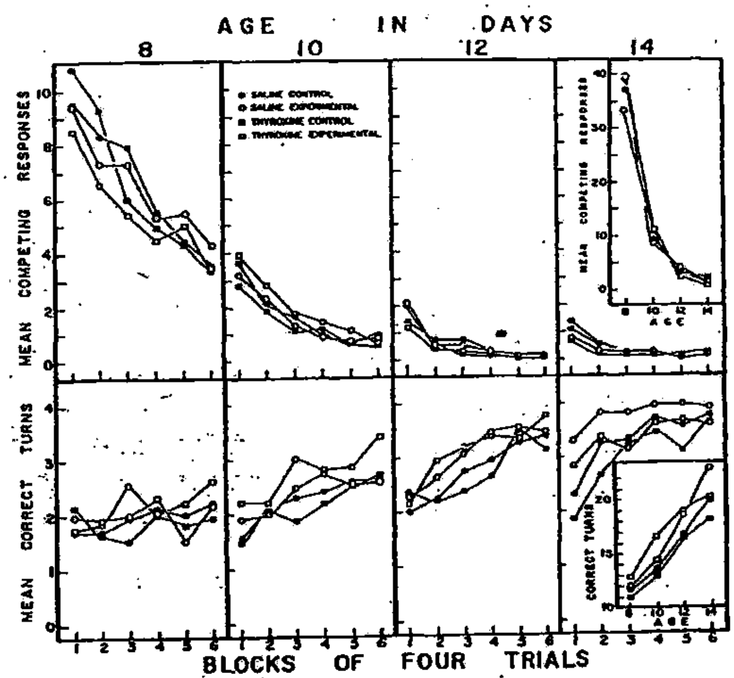

FIGURE 3. The upper panels present the mean number of competing responses made before reaching the choice point, and the lower panels present the mean number of correct choice-point turns during T-maze retention testing as a function of hormone treatment, previous training (experimental groups received previous T-maze training; control groups received previous straight-alley training), age, and blocks of four trials. (Means for the entire retest sessions are given in the figure insets as a function of hormone treatment, previous training, and age.)

.0005. The fact that no other differences were obtained for the competing response measure suggests that the SC, TC, SE, and TE groups were equivalent at each age for the escape response in the stem portion of the T-maze.

Additional findings for the choice-point turn data were significant differences between the experimental and control groups, $F(1, 288) = 31.42$, $p < .0005$, and a Group × Age × Trial Blocks interaction, $F(15, 1440) = 2.17$, $p < .01$. These findings are presented in the lower panel of Figure 3, where an overall greater number of correct choice-point turns is evident for the T-maze trained subjects compared with straight-alley-trained controls, as are the different rates of improvement over trial blocks, with the older experimental groups initially showing a higher number of correct choice-point turns compared with the controls. One further finding was a significant Hormone × Age interaction, $F(3, 288) = 3.48$, $p < .025$, reflecting the fact that although thyroxine-treated subjects were generally superior to saline-treated controls at the three youngest ages, they made fewer correct turns at 14 days of age than did saline animals.

Since the purpose of the present study was to determine age-related retention capacities as a function of hormone treatment, individual comparisons were conducted among the correct choice-point turn scores within each of the four ages. At 8 days of age, the TE group made somewhat more correct turns than did the TC controls, $F(1, 288) = 3.68$, $p < .06$. By 10 days of age, the TE group made significantly more correct turns than did all other groups, $Fs(1, 288) \geq 4.71$, $ps < .05$, indicating that the thyroxine-treated mice with prior training on the T-maze were capable of 24-hr retention. The TC, SC, and SE groups failed to differ from one another at 10 days of age, $Fs(1, 288) \leq 2.14$). At 12 days of age, the TE group again made more correct turns than did the TC group, $F(1, 288) = 6.12$, $p < .025$, while the SE group showed the first indication of a 24-hr retention capacity by making more correct turns than did the SC group, $F(1, 288) = 3.49$, $p < .06$. Although both the SE and TE groups were performing better at 14 days of age than were their respective control groups, a performance deficit was evident for the thyroxine-treated mice as both the thyroxine-treated groups (TE and TC) were performing more poorly than were their respective saline counterparts, $Fs(1, 288) \geq 6.63$, $ps < .025$.

Discussion

The results of Experiment 1 clearly demonstrate that the effects of early hyperthyroidism upon physical development and unlearned behaviors in mice are similar to those reported for rats, resulting in less weight gain, earlier eye opening, and accelerated development of adultlike swimming behaviors (Davenport & Gonzalez, 1973; Schapiro et al., 1970). The finding in Experiment 2 that thyroxine-treated mice ran faster than saline controls on straight-alley errorless trials at each age would also suggest an acceleration of locomotor abilities, although the possibility of increased sensitivity to shock as a result of the thyroxine treatment cannot be discounted.

The finding of major interest in the present study was that thyroxine-treated mice exhibited a capability of 24-hr retention of prior T-maze training at least 2 days earlier than did saline controls, suggesting that early hyperthyroidism also accelerates the development of processes involved in certain kinds of memory mechanisms. When retested at 10 days of age, the TE group that had received T-maze training 24 hr earlier now made more correct choice-point turns than did either the thyroxine group with prior straight-alley training or either of the saline control groups. As the other groups failed to differ from one another during retest, the superior performance of the thyroxine-treated group appears to reflect memory of prior training. An alternative explanation for the TE group's superior performance at 10 days of age might be that early thyroxine affects learning rather than retention ability at this age. While this explanation appears plausible since the TE group showed more rapid improvement over the retest session

than did the SE group, thyroxine treatment should also have resulted in more rapid learning for the TC group in comparison with the SC group, which it did not.

The question whether the superior retest performance of the 10-day-old TE group in comparison with the SE group is indicative of an accelerated maturation of memory capabilities or is simply reflecting differences in original learning must be considered. Both the TE and SE groups showed a similar increase in the number of correct turns over the training session at 9 days of age, and there were no significant differences between these groups within the first and last trial blocks. However, the first-to-last trial block improvement was statistically significant for the TE group, but not for the SE group. Therefore, it might be argued that the SE group failed to demonstrate significant 24-hr retention effects since they did not show significant improvement during original training. While this possibility cannot be rejected on the basis of the present data, previous reports from this laboratory (Nagy & Murphy, 1974; Nagy et al., 1976; Nagy & Sandmann, 1973) have shown untreated 9-day-old mice to have similar rates of improvement as the 9-day-old SE group in the present study. While the improvement over trial blocks was statistically significant at 9 days of age in two of these reports, reliable 24-hr retention effects were not indicated in any of the studies. Therefore, the significant differences in retention performance between the TE and SE groups at 10 days of age are interpreted as reflecting differences in memory rather than learning abilities during original training.

The emergence of 24-hr memory at 11–12 days of age for saline-treated mice is in agreement with previous studies by Nagy and co-workers. The fact that both TE and SE groups showed similar improvement during original training at 11 days of age and comparable amounts of retention suggests that although the early hormone treatment acclerated the onset of 24-hr retention capability, it did not later increase the amount retained by the TE group in comparison with the SE group.

At 14 days of age, the SE and TE groups again made more correct turns during retest than did their respective SC and TC controls. However, the fact that both saline groups made significantly more correct turns than did their respective thyroxine groups indicates a performance deficit at this age as a result of the hormone treatment. Schapiro (1968), noting similar paradoxical effects in early thyroxine-treated rats, suggested that the thyroxine-accelerated development of the CNS may prematurely advance the brain through critical ontogenetic stages, resulting in a decrease in subsequent behavioral plasticity. In view of the present data, it may be that a decrease in behavioral plasticity occurs as early as 14 days of age in the thyroxine-treated mouse. While this deficit occurred earlier than Schapiro reported for rats, there is evidence that mice undergo CNS maturation at a faster rate than rats do (e.g., Agrawal & Himwich, 1970; Nagy, Murphy, & Ray, 1975), and thus, the thyroxine-induced deficit would be expected to occur at a relatively earlier age in mice. Alternatively, it may be that the age at which the decrease in behavioral plasticity becomes evident varies with the type and complexity of the learning task. The fact that a similar deficit was not seen in the competing response measure for any of the thyroxine-treated groups lends support to this hypothesis. Whatever the cause, the similarity of subsequent thyroxine-induced performance deficits in both species is obvious.

In conclusion, the results of the present study do not support the viewpoint of Davenport and Gonzalez (1973) that early thyroxine treatment directly affects only the maturation of locomotor functions. While the thyroxine-treated mice were accelerated in the development of several aspects of locomotor abilities, this earlier maturation did not result in their learning or remembering to reach the choice point any more efficiently than saline controls (as indicated by the competing response data), nor did it result in better learning of the correct choice-point turn through 13 days of age. Therefore, differential maturation of locomotor functioning cannot easily account for the reliable retention effects exhibited at 10 days of age by the thyroxine group. Instead, the present data indicate

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The Systemic Use of Procaine in the Treatment of the Elderly: A Review*

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ABSTRACT: This article is a review and evaluation of the world literature on the systemic use of procaine in the treatment of the aging process and the common chronic diseases of later life. Included are data from 285 articles and books, describing treatment in more than 100,000 patients in the past 25 years. Except for a possible antidepressant effect, there is no convincing evidence that procaine (or Gerovital, of which procaine is the major component) has any value in the treatment of disease in older patients. If procaine has an antidepressant effect, there is some likelihood that this accounts for the reports of decreased complaints referable to the musculoskeletal, cardiovascular, endocrine, sexual, gastrointestinal and respiratory systems.

During the past 25 years, a lengthy series of papers on the therapeutic effects of systemically administered procaine has appeared in the European and American literature. The pharmaceutical preparation most commonly used in the reported studies has been Gerovital (GH-3), which is basically a 2 percent solution of procaine. The majority of these publications claim a beneficial effect of procaine in delaying the aging process or in favorably altering the common chronic diseases of middle and later life. A minority of the papers assert that procaine has no such benefits.

So large and complex has this literature become that a detailed and comprehensive review of the subject appears appropriate. The purposes of this paper are to review the pharmacology of procaine, to examine the therapeutic claims made for it in the fields of aging and chronic illness, including psychiatric illness, and to evaluate these claims as carefully as the literature permits. The paper examines the pharmacologic characteristics of procaine, especially in the doses and by the route employed in much of this literature. The effects of procaine on mood, depressive

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states, altered cerebral function, and chronic diseases of middle and later life are reviewed. Summaries and conclusions are presented in the text at points where their inclusion seems most relevant.

In the preparation of this report, we read and evaluated 285 papers. These included 4 papers in Spanish, 5 in Czech or Polish, 5 in Roumanian, 5 in Portuguese, 44 in French, 81 in German, 12 in Italian and 129 in English. Some of these papers were extremely sketchy, some contained no data, and others represented material published earlier in another language. Therefore, we have not cited all of them in our list of references.

PHARMACOLOGIC CHARACTERISTICS OF PROCAINE

Textbooks, such as that of Goodman and Gilman (1) with the definitive data provided by Ritchie et al (2), contain a comprehensive and accurate discussion of the properties of procaine and other local anesthetics. In addition, there have appeared a number of symposia and review articles on the subject; especially noteworthy are those edited by Fink (3) and the recent basic studies of Cohen et al (4, 5).

As a general conclusion, it can be stated that procaine has effects on all types of neural tissues and excitable cells in concentrations of 2–20 mg/ml (hydrochloride salt). It depresses excitability, increases threshold, slows or blocks conduction of action potentials, decreases or abolishes repetitive activity (such as cardiac arrthymias or sensory nerve discharges) and reduces the efficacy of synaptic transmission, usually without producing major alterations in the resting membrane potential. These effects appear to be due to an interaction with conductance channels of membrane cations (Na^+, K^+, Ca^{++}); especially important is blockade of the increase of Na^+ permeability associated with the action potential and its interaction with Ca^{++} effects.

Because procaine is rapidly hydrolyzed in the body by pseudocholinesterase, one must consider the effects of its metabolites, para-aminobenzoic acid (PABA) and diethylaminoethanol (DEAE). No direct effects of PABA have been identified in man. The graying of the hair found in some PABA-deficient animals has no counterpart in man. DEAE has anti-arrhythmic properties similar to those of procaine but is a very weak local anesthetic. Small doses in man may produce a mild euphoric state (1).

Although these generalizations summarize much of the extensive literature on procaine and its metabolites, they are subject to misinterpretation because of the complexity of the studies. The ionized (cationic) form of procaine is generally believed to be the active moiety; however, closely related compounds (e.g., benzocaine) that cannot ionize can produce local anesthesia indistinguishable from that of classic local anesthetics such as procaine. Ritchie (6) stressed that nitrogenous local anesthetics (e.g., procaine) can act in at least two ways, both in the cationic and in free amino forms, although most experiments reveal an interaction with a receptor internal to the cell that interacts with the cationic form (7). Strichartz (8) concluded that all forms of local anesthetics act primarily by preventing the influx of sodium ions (cf. also 2, 9–15). In addition to blocking conduction, local anesthetics block rapid axoplasmic transport (16). Seeman (17) presented a holistic, comprehensive theory of anesthetic action that includes binding of local anesthetics to the passive Na^+ conductance channel. The effect of procaine on sodium conductance is also seen with slices of brain cortex (18). In addition, local anesthetics inhibit evoked release of glutamate from cortex slices (18) and depress cellular respiration of isolated cortical tissue (19).

With the exquisitely sophisticated techniques of fluorescent probes, Cohen et al (5) analyzed the nature of the binding of local anesthetics to membranes, using isolated membrane fractions from the electric organ of Torpedo marmorata. In such preparations, local anesthetics act allosterically on the binding of the natural neural transmitter. This local anesthetic binding can be abolished by treatment of the membrane fragments with detergents; these observations further support the investigator's theory that local anesthetics do not act directly on the cholinergic receptor site but on other sites on the membrane (4). In addition to an action on Na^+ permeability, the local anesthetics compete with or interfere with Ca^{++} action (13, 20). Moreover, in many respects, local anesthetics act similarly to calcium (4, 5, 21).

The delivery of procaine to the central nervous system (CNS) via the blood stream results in effects dependent upon the concentration and sites to which it is distributed. In experimental studies employing brain and spinal cord preparations, the effects of intravenously administered procaine are transient and require doses of over 2 mg/kg as a bolus of a 20 mg/ml solution. The repetitively active sensory-nerve systems are

among the most sensitive neural systems; depression of tooth afferents (22) or muscle spindle afferents (23) requires a procaine blood level of about 0.25 mg/ml. Analogous data have been obtained from CNS studies (24–26). Such effects are short lasting and appear to be related to blood levels; interrupted infusions incremented every 30 minutes can produce moderately sustained effects for a few hours (23).

The application of procaine (or other local anesthetics) directly to neurons or its injection into the nervous system yields results determined primarily by local anesthetic effects *at the site of application*. Although many references (27–29) describe the actions of local anesthetics on the brain, these effects have little if any relation to the actions of local anesthetics given systemically via the blood supply.

The time course of the action of systemically administered procaine appears, quite plausibly, to be correlated with the blood levels and these, in turn, with procaine's metabolism. Procaine is readily absorbed from subcutaneous or intramuscular sites; in fact, this rapid absorption is the major limitation in its extensive utilization as a local anesthetic. Brodie and coworkers (30) demonstrated in 1948 that the direct intravenous infusion in man of 2,000 mg of procaine over 45–125 minutes resulted in maximum blood levels of only 0.00035 mg/ml or less. This infusion procedure resulted in blood levels analogous to those that might be obtained with an intramuscular dose of approximately 10 ml of a 2 percent solution (200 mg) absorbed completely over a period of 10 minutes. These studies also showed that the human being metabolizes procaine at a rate of approximately 20 mg per minute or more. Once the infusion is stopped, the blood levels drop precipitously, whereas the levels of the metabolites decrease with time somewhat more slowly.

Brodie et al (30) also showed simply and conclusively how rapidly procaine disappears in blood, by adding it in vitro to blood samples. Within one or two minutes after the addition of the procaine, the bulk of the sample had been hydrolyzed; thus, hydrolysis of procaine in plasma is extremely rapid.

That procaine acts as a readily reversible inhibitor of brain monoamine oxidase (MAO) was demonstrated in vitro by a decrease in the metabolism of catecholamines (epinephrine or serotonin) and kynuramine. MacFarlane and Besbris (31) found that this action requires concentrations, even under steady-state conditions, of more than 1 mg/ml for 50 percent inhibition. MAO was derived from rat-brain mitochondrial preparations or from brain homogenates; procaine and Gerovital were of limited potency, with concentrations of orders of magnitude greater than the maximum levels obtained in the blood in vivo. Assuming that upon intramuscular administration procaine reaches a brief period of steady-state equilibrium between brain tissue levels and circulating blood, and that there is ready reversibility, the potency of procaine is so low as to be of doubtful significance, even in relation to the peak concentrations obtainable in vivo.

The evidence (32) that Gerovital is a more effective MAO inhibitor than commercial procaine hydrochloride is not convincing, especially since the differences noted were small. The results and conclusions may be due to sample error or additives present in the Gerovital. Hrachovec is cited in MacFarlane's paper (32) as showing that Gerovital inhibits MAO in vivo and that it is a significantly better MAO inhibitor than procaine. However, none of the references cited contain adequate data to support these claims.

The comparison of Gerovital or procaine with iproniazid (32) is not valid, as the author recognized and emphasized in a subsequent publication. The study of MacFarlane and Besbris (31) essentially showed what had already been assumed by many, that procaine is a *weak, reversible* inhibitor of crude MAO preparations. Reversible enzyme inhibition implies that the inhibitor-enzyme complex is readily and rapidly reversible; to demonstrate such inhibition in vivo requires measuring substrate at given times rather than enzyme activity. After enzyme isolation and dilution, any enzyme-inhibitor complex that might have existed in vivo would be expected to have been reversed in the process.

Under optimal conditions, in vivo concentrations of procaine of 10^{-6} M might be reached. At this level, in vitro and under steady-state conditions, Yau (33) obtained 20–40 percent inhibition of mouse-brain MAO. Also, following single intraperitoneal doses of 90 to 180 mg/kg, Yau (33) found only a "slight, but significant" increase in brain serotonin but no significant change in dopamine or norepinephrine, indicating little if any MAO inhibition. No significant changes were found after chronic dosing with *90 mg/kg* of procaine! Conceding that the positive finding might be pertinent, Yau (33) reported that the animals were *sedated* after these doses of procaine — not stimulated, as has been assumed for man. (This